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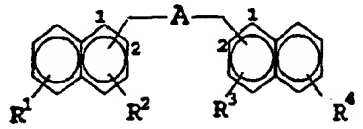

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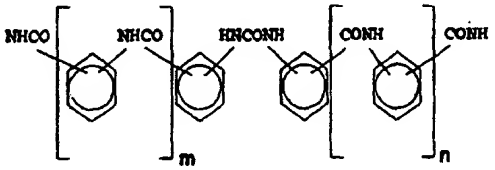

(a)

(57) Abstract

The use of a compound with structural formula (I), wherein R^1 to R^4 are each independently one or more of -X, $-N_3$, $-NO_2$, halo, trifluoromethyl, R^5, OR^5, $-CH_2OR^5$, $-OCOR^5$, $-CH_2OCOR^5$, $-NHCOR^5$, $-CH_2NHCOR^5$, $-NR^5R^6$, $-CH_2NR^5R^6$, $-CH_2NO_2$, $CONR^5R^6$, $CH_2CONR^5R^6$, $-COOR^5$, $-CH_2COOR^5$, -CHO and $-CH_2CHO$ and -X is independently $-SO_3R^5$, $-CH_2PO_3R^5R^6$, $-CH_2SO_3R^5$, $-OSO_3R^5$, $-CH_2OSO_3R^5$, $-CH_2OSO_3R^5$, $-NHSO_3R^5$, $-CH_2NHSO_3R^5$, $-OPO_3R^5R^6$, $-CH_2OPO_3R^5R^6$ and $-PO_3R^5R^6$ where R^5 and R^6 are chosen independently from -H and lower alkyl and wherein A is a chemical group comprising between 5 and 30 bonds directly linking the naphthyl groups provided that (i) the compound is not suramin and (ii) when A is not (a), wherein m and n are independently 0, 1 or 2, then at least one of R^1 to R^4 is -OH or an acidic group or a pharmaceutically acceptable salt, ester, salt of such ester or amide of such compounds, in the manufacture of a medicament for use in reducing undesired angiogenesis, treating cancer, treating fibrotic disease, or treating diseases benefiting from antagonism of the action of fibroblast, vascular endothelial and transforming growth factors. Preferably A is (a) and m and n are independently 0, 1 or 2. Novel pharmaceutical compositions with other medicaments are provided.

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USE OF DINAPHTHALENES COMPOUNDS AS ANTIPROLIFERATIVE AGENTS

The present invention relates to therapeutic compounds with anti-proliferative properties, useful in the treatment of neoplastic disease (ie cancers, tumours) and other conditions, with anti-cancer properties and with anti-fibrotic disease properties, including angiogenesis and other hyperproliferative disorders.

Angiogenesis is the formation of new capillary vessels. It is an important event in embryonic development and in the female reproductive cycle. Pathologically, angiogenesis occurs during the wound-healing process and in a variety of diseases including diabetic retinopathy, psoriasis and several types of chronic inflammation.

The growth of solid tumours is angiogenesis-dependent. Various substances, including growth factors, are known to be involved in tumour-induced angiogenesis by directly and/or indirectly stimulating endothelial cell proliferation and/or migration. One such angiogenic factor is basic Fibroblast Growth Factor (bFGF) whose presence in a large number of normal and malignant cells has been implicated as a factor in both physiological and pathological angiogenesis.

Microvascular endothelial proliferation is thought to be a key event in the complex process of tumour angiogenesis. Other steps include endothelial cell migration, tube formation and anastomoses.

Since solid tumour growth and progression are strictly dependent on capillary formation, interfering with this process by counteracting the effect of angiogenic growth factors may represent a selective therapeutic approach to malignancy.

Suramin is a polysulphonated naphthylurea that has been employed in the treatment of onchocerciasis and trypanosomiasis for over 50 years. Recently, it has been observed that suramin is able, in some cases, to promote a striking remission of Kaposi's sarcoma in AIDS patients. Further studies have shown that suramin has some activity in the treatment of metastatic cancer, particularly metastatic adrenocortical carcinoma. The effectiveness in the treatment of adrenocortical carcinoma is thought to be related to its unusual toxicity towards the adrenal cortex. However, suramin has also been used in the treatment of cancers that are unresponsive to conventional chemotherapy including prostate carcinomas and lymphomas. Suramin administered intraperitoneally has been shown to inhibit growth of human osteosarcoma xenografts in mice for periods of up to nine weeks.

It has been shown by Jentsch *et al* (1987) *J. gen. Virol.* 68, 2183-2192 that various suramin-related compounds inhibit human immunodeficiency virus type I (HIV-I) reverse transcriptase.

In vitro, suramin has been shown to block the growth-stimulating activity of several growth factors including platelet-derived growth factor; epidermal growth factor; transforming growth factor-β; insulin-like growth factor-1; and growth factors for endothelial cells, including members of the fibroblast growth factor (FGF) family and vascular endothelial growth factor.

The aforementioned studies have shown that suramin is able to block the binding of growth factors to their receptors in intact cells either by binding to the growth factor itself, or possibly to the growth factor receptor. Additionally, suramin has other features which could account for its anti-proliferative and anti-metastatic activities. These include, in the context

of anti-proliferation, inhibition of key enzymes involved in the intracellular transduction of mitogenic signals.

The major limitation in the clinical use of suramin in the treatment of tumours is the narrow margin between serious toxicity, and the level required to achieve anti-tumour activity. The most prevalent side-effect is adrenal failure arising from damage to the cortex. Suramin toxicity has been reviewed recently.

It has been known for many years that very small variations in the structure of the suramin molecule may lead to a significant decline in activity. For example, replacement of the two methyl groups of suramin with hydrogen results in a compound (CPD16; Figure 1) with only about 5% of the trypanocidal activity of suramin (Fourneau *et al* (1924) *Annales de L'Institut Pasteur* **38**, 81). Nakajima *et al* disclosed (Nakajima *et al* (1991) *J. Biol. Chem.* **266**, 9661-9666) that trypan blue and Evans blue, both polysulphonated compounds structurally related to suramin, are at least six times less active than suramin in their ability to inhibit heparinase and inhibit BI6 melanoma cell traversing an extracellular "Matrigel" matrix in an "invasion" assay.

Other polyanions, unrelated to suramin, have been used to inhibit cell growth, including that of endothelial cells. For example, pentosan polysulphate (PPS) is a heparin analogue that inhibits K-FGF stimulated growth of SW13 adrenocortical cells transfected with the K-FGF gene; PPS exhibited selective inhibition of K-FGF induced proliferation by a factor of 2000-fold, compared to inhibition by suramin and dextran sulphate of 3- and 5-fold respectively (Wellstein *et al* (1991) *J. Natl. Cancer Inst.* **83**, 716-720).

WO 90/15816 discloses the use of suramin, angiostatic steroids and various naphthalene sulphonic acids for treating angiogenesis.

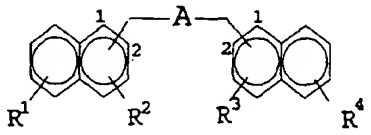
WO 91/12009 discloses the use of suramin, heparin sulphate, dextran sulphate and polysulphonated glycosaminoglycans for treating rheumatoid arthritis.

WO 91/13624 discloses the use of sulphated glycosamino glycan, such as heparan sulphate, dermatan sulphate, chondroitin sulphate and keratan sulphate, sometimes in combination with suramin, for treating cancer.

WO 93/07864 discloses the use of suramin and other polysulphonated compounds as a contraceptive agent.

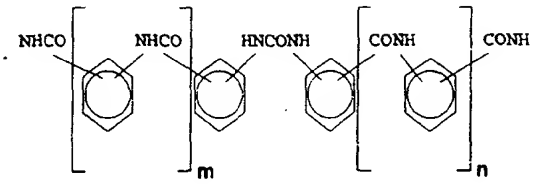
The present invention relates to the use of dinaphthylene compounds in medicine.

A first aspect of the present invention provides the use of a compound with the structural formula:

(I) 

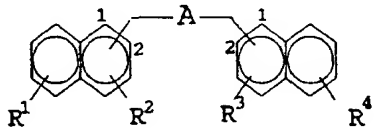
wherein R^1 to R^4 are each independently one or more of -X, $-N_3$, $-NO_2$, halo, trifluoromethyl, R^5, OR^5, $-CH_2OR^5$, $-OCOR^5$, $-CH_2OCOR^5$, $-NHCOR^5$, $-CH_2NHCOR^5$, $-NR^5R^6$, $-CH_2NR^5R^6$, $-CH_2NO_2$, $CONR^5R^6$, $CH_2CONR^5R^6$, $-COOR^5$, $-CH_2COOR^5$, -CHO and $-CH_2CHO$ and wherein A is a chemical group comprising at least 5 and no more than 30 bonds

directly linking the naphthyl groups provided that (i) the compound is not suramin and (ii) when A is not

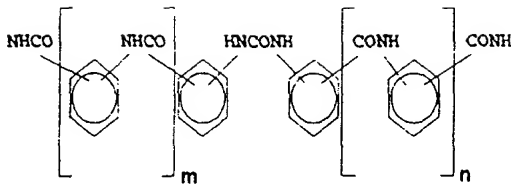


wherein m and n are independently 0, 1 or 2, then at least one of R^1 to R^4 is -OH or an acidic group; or a pharmaceutically acceptable salt, ester, salt of such ester or amide of such compounds, in the manufacture of a medicament for use in treating cancer.

A second aspect of the invention provides a compound with structural formula:

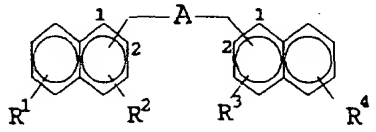
(I) 

wherein R^1 to R^4 are each independently one or more of -X, $-N_3$, $-NO_2$, halo, trifluoromethyl, R^5, OR^5, $-CH_2OR^5$, $-OCOR^5$, $-CH_2OCOR^5$, $-NHCOR^5$, $-CH_2NHCOR^5$, $-NR^5R^6$, $-CH_2NR^5R^6$, $-CH_2NO_2$, $CONR^5R^6$, $CH_2CONR^5R^6$, $-COOR^5$, $-CH_2COOR^5$, -CHO and $-CH_2CHO$ and wherein A is a chemical group comprising at least 5 and no more than 30 bonds directly linking the naphthyl groups provided that (i) the compound is not suramin and (ii) when A is not

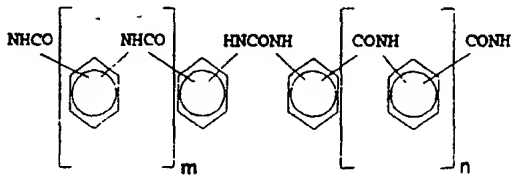


wherein m and n are independently 0, 1 or 2, then at least one of R^1 to R^4 is -OH or an acidic group;or a pharmaceutically acceptable salt, ester, salt of such ester or amide of such compounds, in the manufacture of a medicament for use in reducing undesired angiogenesis.

A third aspect of the invention provides a compound with structural formula:

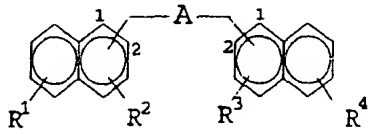
(I) 

wherein R^1 to R^4 are each independently one or more of -X, $-N_3$, $-NO_2$, halo, trifluoromethyl, R^5, OR^5, $-CH_2OR^5$, $-OCOR^5$, $-CH_2OCOR^5$, $-NHCOR^5$, $-CH_2NHCOR^5$, $-NR^5R^6$, $-CH_2NR^5R^6$, $-CH_2NO_2$, $CONR^5R^6$, $CH_2CONR^5R^6$, $-COOR^5$, $-CH_2COOR^5$, -CHO and $-CH_2CHO$ and wherein A is a chemical group comprising at least 5 and no more than 30 bonds directly linking the naphthyl groups provided that (i) the compound is not suramin and (ii) when A is not

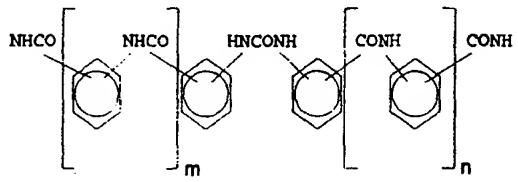


wherein m and n are independently 0, 1 or 2, then at least one of R^1 to R^4 is -OH or an acidic group; or a pharmaceutically acceptable salt, ester, salt of such ester or amide of such compounds, in the manufacture of a medicament for use in treating fibrotic diseases.

A fourth aspect of the invention provides a compound with structural formula:

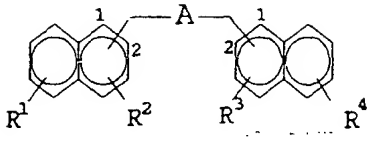
(I) 

wherein R^1 to R^4 are each independently one or more of -X, $-N_3$, $-NO_2$, halo, trifluoromethyl, R^5, OR^5, $-CH_2OR^5$, $-OCOR^5$, $-CH_2OCOR^5$, $-NHCOR^5$, $-CH_2NHCOR^5$, $-NR^5R^6$, $-CH_2NR^5R^6$, $-CH_2NO_2$, $CONR^5R^6$, $CH_2CONR^5R^6$, $-COOR^5$, $-CH_2COOR^5$, -CHO and $-CH_2CHO$ and wherein A is a chemical group comprising at least 5 and no more than 30 bonds directly linking the naphthyl groups provided that (i) the compound is not suramin and (ii) when A is not

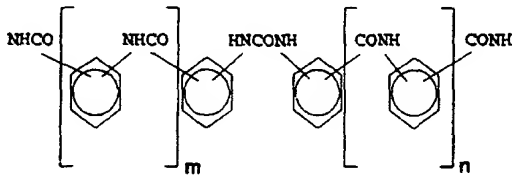


wherein m and n are independently 0, 1 or 2, then at least one of R^1 to R^4 is -OH or an acidic group; or a pharmaceutically acceptable salt, ester, salt of such ester or amide of such compounds, in the manufacture of a medicament for use in treating non-malignant hyper-proliferative diseases.

A fifth aspect of the invention provides a compound with structural formula:

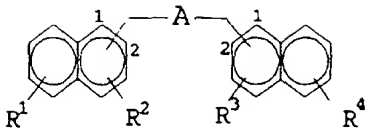
(I) 

wherein R^1 to R^4 are each independently one or more of -X, $-N_3$, $-NO_2$, halo, trifluoromethyl, R^5, OR^5, $-CH_2OR^5$, $-OCOR^5$, $-CH_2OCOR^5$, $-NHCOR^5$, $-CH_2NHCOR^5$, $-NR^5R^6$, $-CH_2NR^5R^6$, $-CH_2NO_2$, $CONR^5R^6$, $CH_2CONR^5R^6$, $-COOR^5$, $-CH_2COOR^5$, -CHO and $-CH_2CHO$ and wherein A is a chemical group comprising at least 5 and no more than 30 bonds directly linking the naphthyl groups provided that (i) the compound is not suramin and (ii) when A is not

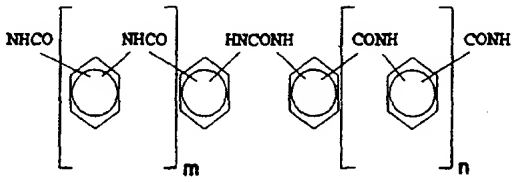


wherein m and n are independently 0, 1 or 2, then at least one of R^1 to R^4 is -OH or an acidic group; or a pharmaceutically acceptable salt, ester, salt of such ester or amide of such compounds, in the manufacture of a medicament for use in treating diseases which benefit from the antagonism of the action of heparin-dependent growth factors.

A sixth aspect of the invention provides a compound with structural formula:

(I) 

wherein R^1 to R^4 are each independently one or more of -X, $-N_3$, $-NO_2$, halo, trifluoromethyl, R^5, OR^5, $-CH_2OR^5$, $-OCOR^5$, $-CH_2OCOR^5$, $-NHCOR^5$, $-CH_2NHCOR^5$, $-NR^5R^6$, $-CH_2NR^5R^6$, $-CH_2NO_2$, $CONR^5R^6$, $CH_2CONR^5R^6$, $-COOR^5$, $-CH_2COOR^5$, -CHO and $-CH_2CHO$ and wherein A is a chemical group comprising at least 5 and no more than 30 bonds directly linking the naphthyl groups provided that (i) the compound is not suramin and (ii) when A is not



wherein m and n are independently 0, 1 or 2, then at least one of R^1 to R^4 is -OH or an acidic group; or a pharmaceutically acceptable salt, ester, salt of such ester or amide of such compounds, in the manufacture of a medicament for use in treating restenosis.

The following definitions and preferences apply to the first, second, third, fourth, fifth and sixth aspects of the invention.

By "halo" we mean bromo, chloro, fluoro or iodo.

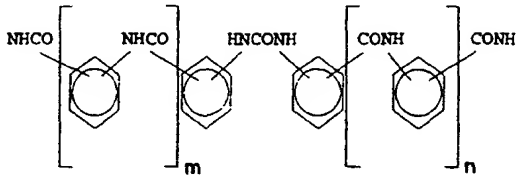
By "acidic group" we include $-SO_3H$, -COOH, $-OSO_3H$, $-CH_2PO_3H_2$ and $-PO_3H_2$.

X is independently $-SO_3R^5$, $-CH_2PO_3R^5R^6$, $-CH_2SO_3R^5$, $-OSO_3R^5$, $-CH_2OSO_3R^5$, $-CH_2OSO_3R^5$, $-NHSO_3R^5$, $-CH_2NHSO_3R^5$, $-OPO_3R^5R^6$, $-CH_2OPO_3R^5R^6$ and $-PO_3R^5R^6$. R^5 and R^6 are chosen independently from -H and lower alkyl. By "lower alkyl" we include $-CH_3$, $-C_2H_5$, $-C_3H_7$, $-C_4H_9$, $-C_5H_{11}$ and $-C_6H_{13}$.

In one embodiment the linkage of A to the naphthyl ring is *via* an amino group.

In another embodiment the linkage of A to the naphthyl ring is *via* an amide or sulphonamide group.

It is preferred if A is:



and m and n are independently 0, 1 or 2, and in further preference wherein n = m = 0 or 1.

In a further embodiment A is selected from the group consisting of straight chain or branched alkyl groups, aryl groups, alkylaryl groups, aliphatic dicarboxylic acids, polyenes and derivatives thereof and polyols and derivatives thereof. Preferably, the number of atoms not on the direct link between the naphthyl groups does not exceed 200 and preferably it is below 100 or 50. It is also preferable for the length of any single branch (eg alkyl, aryl etc) off the direct connection not to exceed 50 atoms.

In a still further embodiment A is an oligopeptide.

In a still further embodiment A is a carbohydrate.

It is preferred if A contains no amino or -SH group or other reactive group that may interfere with synthesis or render the molecule chemically reactive.

The aminophenylcarboxylate groups bridging the naphthyl groups may be joined by *ortho*, *meta* or *para* linkages. Bridging by *meta* linkage is preferred.

It is preferable to use a compound wherein $R^1 = R^4$ and $R^2 = R^3$. Preferably R^1 to R^4 are independently selected from the group consisting of X, -H, lower alkyl, -OH, $-CH_2OH$, $-NHCOCH_3$, $-CH_2NHCOCH_3$, $-CONHCH_3$ and $-CH_2CONHCH_3$. Preferably X is independently selected from the group consisting of $-SO_3H$, $-CH_2SO_3H$, -COOH, $-CH_2COOH$, $-NHSO_3H$, $-CH_2NHSO_3H$, $-OSO_3H$ and $-CH_2OSO_3H$. Preferred compounds are disclosed in Figure 1.

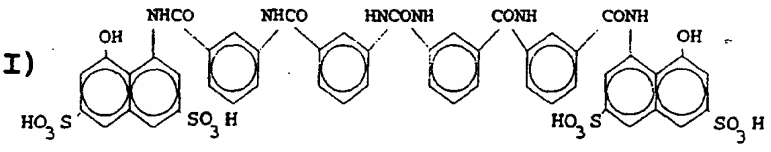
It is still more preferable to use a compound wherein there are one, two or three R^1 groups selected independently from -X, and -OH and one, two or three R^4 groups selected independently from -X, and -OH and R^2 and R^3 is hydrogen or 3-X. It is further preferred to use compounds wherein one each of the R^1 and R^4 groups is -OH at position 8. It is still further preferred to use compounds wherein one each of the R^2 and R^3 groups is -X at position 3 or 4. It is still more preferred to use compounds wherein one each of the R^1 and R^4 groups are -X at position 6 and one each of the R^2 and R^3 groups are -X at position 3. It is still more preferred to use compounds wherein two each of the R^1 and R^4 groups are -X at positions 5 and 7. It is preferred if the -X is $-SO_3H$.

A further preference is to use compounds wherein the naphthyl rings are monohydroxy, disulphonate substituted or are monosulphonate, dihydroxy substituted. Substitution of 8-hydroxy-3,6-disulphonate is most preferred.

Such compounds of the invention have been found to have a therapeutic advantage over suramin as anti-proliferative agents (as indicated in Table I).

In further preference, use of any of the compounds:

(III)



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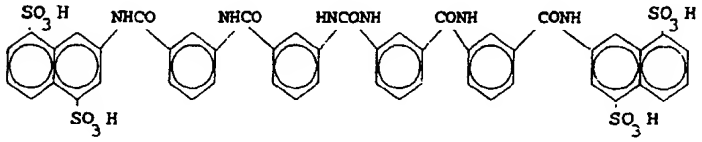
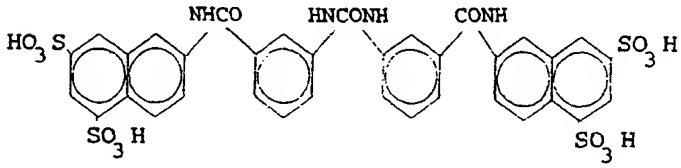


(V)



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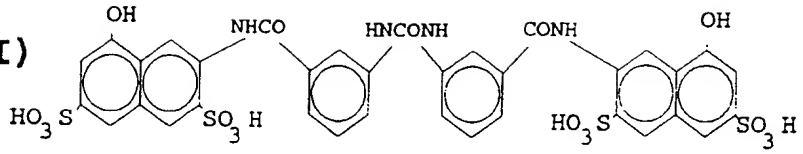

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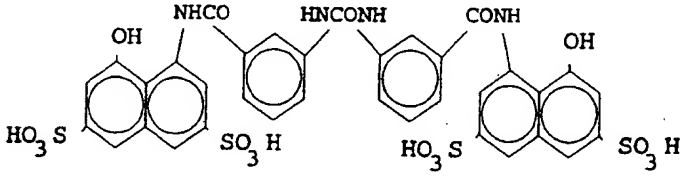

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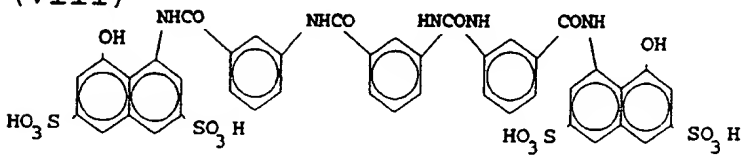

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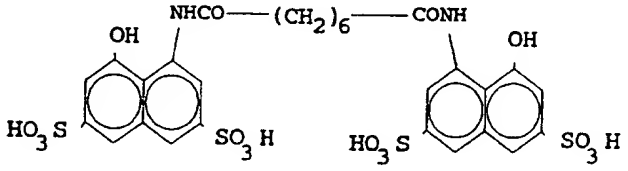

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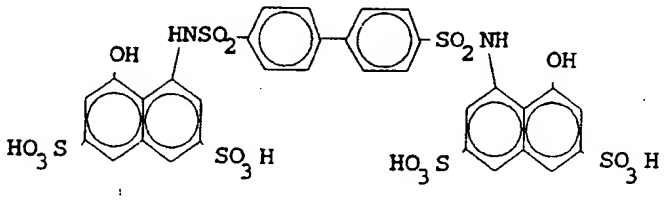

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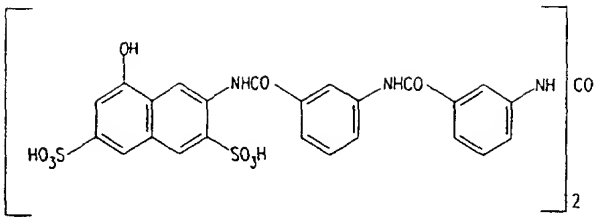


has been found to provide a therapeutic index (measured as defined below) greater than 25 in the case of CPD8 (III; TI = 26.2) and CPD11 (IV; TI = 26.8) and greater than 80 in the case of CPD12 (V; TI = 86.7) and CPD14 (VI; TI = 83.1). The compound labelled VII is N5; the compound labelled VIII is N6; the compound labelled IX is N7; the compound labelled X is N8. The highest TI values are with compounds that have two bridging rings (CPD12 and CPD14). This is largely because of equipotent growth inhibition to that of four bridging ring compounds but reduced toxicity.

Salts which may be conveniently used in therapy include physiologically acceptable base salts, for example, derived from an appropriate base, such as an alkali metal (eg sodium), alkaline earth metal (eg magnesium) salts, ammonium and NX_4^+ and NHX_3^+ and $NH_2X_2^+$ (wherein X is C_{1-4} alkyl) salts. Physiologically acceptable acid salts include hydrochloride, sulphate, mesylate, besylate, phosphate and glutamate. Salts according to the invention may be prepared in conventional manner, for example by reaction of the parent compound with an appropriate base to form the corresponding base salt, or with an appropriate acid to form the corresponding acid salt. Sulphonate esters according to the invention, such as alkyl- or aryl-sulphonyl (eg methyl sulphonyl) may be prepared in conventional manner, for example, by treatment of the parent compound with an appropriate esterifying agent. Amides according to the invention may be prepared in conventional manner, for example by treatment of the

parent compound with an appropriate carboxylic acid or carbonyl halide. In the first aspect of the invention by "cancer" we include sarcomas, leukaemias, lymphomas and cancers of the uterine cervix, head, neck, brain gliomas, breast (including hormone-refractory), colon, rectum, stomach, bladder, lung, prostate (including hormone refractory), skin (including Kaposi's sarcoma), mouth, nose, oesophagus, stomach, liver, pancreas, and metastatic forms of any of these. It is preferred if the cancer to be treated is a solid tumour or a solid metastatic form of a cancer.

In the second aspect of the invention by "reducing undesired angiogenesis" we mean reducing to a useful extent the formation of new capillary vessels in a patient where such new capillary vessel formation is clinically undesirable or is otherwise unwanted. There are many diseases in which it is desirable to reduce undesired angiogenesis (which contributes to the disease) including cancers in which angiogenesis plays a role, retinopathy including diabetic retinopathy, psoriasis and chronic inflammations including rheumatoid arthritis. By "cancer in which angiogenesis plays a role" we include solid tumours for example prostate cancer, breast cancer and ovarian cancer, and also lymphoma.

Angiogenesis occurs during the normal menstrual cycle; in some cases this may be undesirable and the compounds of the invention are believed to be useful as a female contraceptive.

In the third aspect of the invention by "fibrotic diseases" we mean diseases that are characterised by fibrotic reactions in vital organs. Proliferation of fibroblasts and stimulation of extracellular matrix production by the stromal cells characterises, but is not limited to, the following fibrotic diseases: pulmonary fibrosis, retroperitoneal fibrosis,

scleroderma, cirrhosis of the liver, fibrosing syndromes include mediastinal fibrosis, sclerosing cholangitis, Riedel's thyroiditis, pseudo tumour of the orbit, Peyronie's disease, chronic pancreatitis, Crohn's disease, endocardial fibroelastosis, endomyocardial fibrosis and glomerulonephritis.

In the sixth aspect of the invention by "restenosis" we mean the condition arising subsequent to a surgical procedure which has been performed for the purpose of improving blood flow in an atherosclerotic artery, such condition being characterised by smooth muscle cell proliferation and intimal wall thickening leading to reclosure of the artery. This would include but not be limited to such surgical procedures as balloon angioplasty, coronary artery bypass graft surgery, and the engraftment of artificial vessels. The compounds of the invention may antagonise the action of a growth factor. It is preferred if the growth factor is a heparin-binding growth factor or a growth factor dependent on a heparin-binding protein. It is further preferred if the growth factor is selected from the group consisting of fibroblast growth factor (FGF), insulin-like growth factor-1 (IGF-1), platelet-derived growth factor (PDGF), vascular endothelial growth factor (VEGF), transforming growth factor β (TGFβ) HB-GF and pleiotropin. Thus the compounds of the invention are useful in treating diseases in which such antagonising is desirable. Benign proliferations and hypertrophy of cells caused by such growth factors include benign prostatic hypertrophy and fibromuscular hyperplasia of large vessels (predominantly extracranial and renal vessels) and the compounds of the invention are useful in treating such conditions.

The compounds of the invention are useful for inhibiting endothelial cell proliferation including the proliferation of cells of the microvascular endothelium and large vessel endothelium, and also in inhibiting the

growth of epithelial cells.

Thus, further aspects of the invention are methods of treating mammals benefiting from reducing undesired angiogenesis, methods of treating patients with cancer, methods of treating patients with fibrotic disease, methods of treating patients at risk for developing restenosis, and methods of treating patients benefiting from the antagonism of the action of growth factors, the methods comprising administering to the said mammal or patient an effective dose or doses of the compounds defined in the first aspect of the invention.

When treating patients, cells may die through the programmed cell death pathway of apoptosis. Thus, the compounds may be useful in causing apoptosis.

Suitable doses of the compounds are between 10 μg/kg of body weight and 1 g/kg of body weight when administered systemically; it is preferred if the dose is between 100 μg/kg and 500 mg/kg, more preferably between 500 μg/kg and 200 mg/kg, more preferably still between 1 mg/kg and 100 mg/kg and in still further preference between 10 mg/kg and 80 mg/kg. It will be appreciated that the dose may be varied in a non-inventive way by the physician responsible for administration. Formulations and methods of administration are described below. Doses of the compound for topical application may be between 1 ng/cm^2 body surface area and 1 mg/cm^2 body surface; it is preferred if the dose is between 10 ng/cm^2 and 100 μg/cm^2, more preferably between 100 ng/cm^2 and 10 μg/cm^2.

Still further aspects of the invention are a method of inhibiting endothelial cell proliferation, the method comprising the step of exposing the said endothelial cell to a compound as defined in the first aspect of the

invention; a method of inhibiting growth factor-stimulated cell proliferation, the method comprising the step of exposing the cell to a compound as defined in the first aspect of the invention; and a method of inhibiting epithelial cell proliferation, the method comprising the step of exposing the said epithelial cell to a compound as defined in the first aspect of the invention. It is preferred if the growth-factor stimulated cells are those that are stimulated by heparin-dependent growth factors, for example PDGF, TGF-β, IGF-1, FGF including basic FGF, and VEGF.

Mammals to be treated include humans, cats, dogs, pigs, horses, cattle and sheep.

By "treating" a disease we mean ameliorating an existing disease, or preventing a potential disease, to a useful extent.

The aforementioned compounds of the invention or a formulation thereof may be administered by any conventional method including oral and parenteral (eg subcutaneous or intramuscular) injection. The treatment may consist of a single dose or a plurality of doses over a period of time.

Whilst it is possible for a compound of the invention to be administered alone, it is preferable to present it as a pharmaceutical formulation, together with one or more acceptable carriers. The carrier(s) must be "acceptable" in the sense of being compatible with the compound of the invention and not deleterious to the recipients thereof.

The formulations may conveniently be presented in unit dosage form and may be prepared by any of the methods well known in the art of pharmacy. Such methods include the step of bringing into association the active ingredient (compound of the invention) with the carrier which

constitutes one or more accessory ingredients. In general the formulations are prepared by uniformly and intimately bringing into association the active ingredient with liquid carriers or finely divided solid carriers or both, and then, if necessary, shaping the product.

Formulations in accordance with the present invention suitable for oral administration may be presented as discrete units such as capsules, cachets or tablets, each containing a predetermined amount of the active ingredient; as a powder or granules; as a solution or a suspension in an aqueous liquid or a non-aqueous liquid; or as an oil-in-water liquid emulsion or a water-in-oil liquid emulsion. The active ingredient may also be presented as a bolus, electuary or paste.

A tablet may be made by compression or moulding, optionally with one or more accessory ingredients. Compressed tablets may be prepared by compressing in a suitable machine the active ingredient in a free-flowing form such as a powder or granules, optionally mixed with a binder (eg povidone, gelatin, hydroxypropylmethyl cellulose), lubricant, inert diluent, preservative, disintegrant (eg sodium starch glycollate, cross-linked povidone, cross-linked sodium carboxymethyl cellulose), surface-active or dispersing agent. Moulded tablets may be made by moulding in a suitable machine a mixture of the powdered compound moistened with an inert liquid diluent. The tablets may optionally be coated or scored and may be formulated so as to provide slow or controlled release of the active ingredient therein using, for example, hydroxypropylmethylcellulose in varying proportions to provide desired release profile.

Formulations suitable for topical administration in the mouth include lozenges comprising the active ingredient in a flavoured basis, usually sucrose and acacia or tragacanth; pastilles comprising the active ingredient

in an inert basis such as gelatin and glycerin, or sucrose and acacia; and mouth-washes comprising the active ingredient in a suitable liquid carrier.

Formulations suitable for parenteral administration include aqueous and non-aqueous sterile injection solutions which may contain anti-oxidants, buffers, bacteriostats and solutes which render the formulation isotonic with the blood of the intended recipient; and aqueous and non-aqueous sterile suspensions which may include suspending agents and thickening agents. The formulations may be presented in unit-dose or multi-dose containers, for example sealed ampoules and vials, and may be stored in a freeze-dried (lyophilised) condition requiring only the addition of the sterile liquid carrier, for example water for injections, immediately prior to use. Extemporaneous injection solutions and suspensions may be prepared from sterile powders, granules and tablets of the kind previously described.

Preferred unit dosage formulations are those containing a daily dose or unit, daily sub-dose or an appropriate fraction thereof, of an active ingredient.

It should be understood that in addition to the ingredients particularly mentioned above the formulations of this invention may include other agents conventional in the art having regard to the type of formulation in question, for example those suitable for oral administration may include flavouring agents.

The compounds of the invention are useful in treating undesired angiogenesis. One such form of undesired angiogenesis occurs during the growth of solid tumours. The compounds of the invention may be useful in limiting growth of the said solid tumour by reducing its blood supply

and causing necrosis. The compounds may also be useful in preventing the establishment of secondary tumours following metastasis from either solid or non-solid tumours, by preventing the growth of capillary vessels which are required to provide the said secondary tumours with a sufficient blood supply. Suitable formulations of the compound of the invention may be injected directly into the tumour, or may be administered orally, intravesically (in the case of bladder tumours), intraperitoneally, or by any other of the well known routes.

A further form of angiogenesis, which may be undesired if conception is to be prevented, occurs during the female reproductive cycle when the endometrium is formed. Administration of the compound of the invention orally, or by depot injection, may be used to interfere with the menstrual cycle, thus providing a use as a contraceptive agent.

The compounds of the invention are further useful in directly controlling the undesired proliferation of cancer cells in a primary or a metastatic tumour (particularly when the tumour is a cancer of the prostate, breast, stomach, ovary, and the like).

The compounds of the invention are further useful in controlling the proliferation of smooth muscle cells and other cells of the intima which may occur subsequent to surgical procedures involving the vasculature, especially the heart.

Further aspects of the invention provide compositions comprising a compound as defined in the second aspect of the invention in combination with one or more ingredients used in female contraceptives; compositions comprising a compound as defined in the first aspect of the invention in combination with one or more cancer therapeutic agents: and compositions

comprising a compound as defined in any aspect of the invention in combination with one or more symptom-alleviating co-factors such as an antibiotic, anti-inflammatory agent, analgesic or anaesthetic.

Female contraceptives suitable for combination with the compounds include ethynodiol diacetate, ethinylestradiol, norethynodrel, mestranol, norethindrone, lynoestrenol, desogestrel, levonorgestrel, gestodene, norethisterone, norethisterone enanthate, medroxyprogesterone acetate, norethindrone acetate, norgestrel, dimethistrone and diethylstilbestrol. Such combinations provide novel female contraceptives and may be claimed as such herein.

Suitable cancer chemotherapeutic agents for use in combination with the compounds of the invention include: alkylating agents including nitrogen mustards such as mechlorethamine (HN_2), cyclophosphamide, ifosfamide, melphalan (L-sarcolysin) and chlorambucil; ethylenimines and methylmelamines such as hexamethylmelamine, thiotepa; alkyl sulphonates such as busulfan; nitrosoureas such as carmustine (BCNU), lomustine (CCNU), semustine (methyl-CCNU) and streptozocin (streptozotocin); and triazenes such as decarbazine (DTIC; dimethyltriazenoimidazole-carboxamide); Antimetabolites including folic acid analogues such as methotrexate (amethopterin); pyrimidine analogues such as fluorouracil (5-fluorouracil; 5-FU), floxuridine (fluorodeoxyuridine; FUdR) and cytarabine (cytosine arabinoside); and purine analogues and related inhibitors such as mercaptopurine (6-mercaptopurine; 6-MP), thioguanine (6-thioguanine; TG) and pentostatin (2'-deoxycoformycin). Natural Products including vinca alkaloids such as vinblastine (VLB) and vincristine; epipodophyllotoxins such as etoposide and teniposide; antibiotics such as dactinomycin (actinomycin D), daunorubicin (daunomycin; rubidomycin), doxorubicin, bleomycin, plicamycin

(mithramycin) and mitomycin (mitomycin C); enzymes such as L-asparaginase; and biological response modifiers such as interferon alphenomes. Miscellaneous agents including platinum coordination complexes such as cisplatin (*cis*-DDP) and carboplatin; anthracenedione such as mitoxantrone and anthracycline; substituted urea such as hydroxyurea; methyl hydrazine derivative such as procarbazine (N-methylhydrazine, MIH); and adrenocortical suppressant such as mitotane (*o,p'*-DDD) and aminoglutethimide; taxol and analogues/derivatives; and hormone agonists/antagonists such as flutamide and tamoxifen.

Symptom alleviating co-factors for use in combination with the compounds of the invention include: antibiotics such as anti-bacterial agents, for example natural and synthetic penicillins and cephalosporins, sulphonamides, erythromycin, kanomycin, tetracycline, chloramphenicol, rifampicin and including gentamicin, ampicillin, benzypenicillin, benethamine penicillin, benzathine penicillin, phenethicillin, phenoxymethyl penicillin, procaine penicillin, cloxacillin, flucloxacillin, methicillin sodium, amoxicillin, bacampicillin hydrochloride, ciclacillin, mezlocillin, pivampicillin, talampicillin hydrochloride, carfecillin sodium, piperacillin, ticarcillin, mecillinam, pirmecillinan, cefaclor, cefadroxil, cefotaxime, cefoxitin, cefsulodin sodium, ceftazidime, ceftizoxime, cefuroxime, cephalexin, cephalothin, cephamandole, cephazolin, cephradine, latamoxef disodium, aztreonam, chlortetracycline hydrochloride, clomocycline sodium, demeclocydine hydrochloride, doxycycline, lymecycline, minocycline, oxytetracycline, amikacin, framycetin sulphate, neomycin sulphate, netilmicin, tobramycin, colistin, sodium fusidate, polymyxin B sulphate, spectinomycin, vancomycin, calcium sulphaloxate, sulfametopyrazine, sulphadiazine, sulphadimidine, sulphaguanidine, sulphaurea, capreomycin, metronidazole, tinidazole, cinoxacin, ciprofloxacin, nitrofurantoin, hexamine, streptomycin, carbenicillin,

colistimethate, polymyxin B, furazolidone, nalidixic acid, trimethoprim-sulfamethoxazole, clindamycin, lincomycin, cycloserine, isoniazid, ethambutol, ethionamide, pyrazinamide and the like; anti-fungal agents, for example miconazole, ketoconazole, itraconazole, fluconazole, amphotericin, flucytosine, griseofulvin, natamycin, nystatin, and the like; and anti-viral agents such as acyclovir, AZT, ddI, amantadine hydrochloride, inosine pranobex, vidarabine, and the like.

Anti-inflammatory and analgesic agents include acetylsalicylic acid, indomethacin, ibuprofen, acetaminophen, phenacetin, paracetamol, choline magnesium trisalicylate, salsalate, sodium salicylate, azapropazone, diclofenac sodium, diflunisal, etodolac, fenbufen, fenoprofen, flurbiprofen, indomethacin, ketoprofen, mefenamic acid, nabumetone, naproxen, phenylbutazone, piroxicam, sulindac, tiaprofenic acid, tolmetin and the like.

Analgesic agents include morphine, codeine, hydromorphone, naloxone, naltrexone, buprenorphine, dextromoramide, dextropropoxyphene hydrochloride, diamorphine hydrochloride, dihydrocodeine tartrate, dipipanone hydrochloride, levorphanol tartrate, meptazinol, methadone hydrochloride, nalbuphine hydrochloride, pentazocine, pethidine hydrochloride, phenazocine hydrobromide and the like.

Anaesthetic agents include diazepam, lorazepam, promethazine hydrochloride, temazepam, trimeprazine tartrate and the like.

These combinations provide novel medicines for the treatment of the aforementioned diseases and are believed to be of particular benefit to the patients so treated.

Other specific areas where a therapeutically beneficial interaction is expected using the compounds of the invention are now given.

Interaction with hypoxically activated drugs. Drugs activated under hypoxia include mitomycin C, SR4233, RB6145, porfiromycin, EO9. By depriving tumours of their blood supply hypoxically activated drugs or drugs activated by bioreduction may be expected to have increased therapeutic activity and thus may be used in combination with the compounds of the invention.

Combinations of antiangiogenic drugs. Angiogenesis is a complex process with many different mechanisms of action involved in its inhibition thus a combination of the compounds of the invention with AGM1470 and other antiangiogenic drugs may be useful.

Combination with hormonal agents. Hormone sensitive tumours and tumours that become resistant to hormone therapy can be treated with antiandrogen, antioestrogens, antiprogestogens or antiglucocorticoids and their analogues in combination with the compounds of the invention.

Embolisation and photodynamic therapy. Embolisation is a standard treatment of liver secondaries and primary liver tumours and is applicable to deposits from colorectal cancer and hormone producing endocrine tumours. The problem is the regrowth of new vessels after embolisation and the compounds of the invention are useful as a maintenance therapy to prevent this happening.

Photodynamic therapy (PDT), a useful tumour treatment, damages the vasculature of tumours but new blood vessels may regrow, thus maintenance therapy with the compounds of the invention may help

prevent this, and a combination of the compounds with PDT is desirable.

Regional perfusion of limbs. Perfusion of limbs with standard chemotherapy agents and cytokines produces marked antitumour responses, partly by damaging vasculature. Prevention of regrowth of vasculature may help maintain remissions and the compounds of the invention are useful as maintenance treatment or in combination with regional perfusion.

Multidrug resistance. Membrane mechanisms may cause rapid efflux of anticancer drugs causing resistance to many types of anticancer agents. The compounds of the invention, which may interact on membrane sites, are useful in combination with other chemotherapeutic agents to reverse drug resistance.

The antitumour effect of angiogenesis inhibitor AGM-1470 is discussed by Toi *et al* (1993) *Int. J. Oncol.* **3**, 525-528 and Yamaoka *et al* (1993) *Cancer Res.* **53**, 4262-4267. Bioreductive drugs and their role in cancer therapy are disclosed in Workman & Stratford (1993) *Cancer and Metastasis Rev.* **12**, 73-82.

The compounds have also found uses *in vitro*. For example, because the compounds have been found to inhibit endothelial cell proliferation *in vitro*, they are useful as a control in screening assays for other compounds with a similar inhibitory effect. Similarly, the compounds of the invention are useful for screening for other compounds that antagonise their effect. Such other compounds may enhance endothelial cell growth and may be useful promoters of angiogenesis.

Chemical syntheses

Synthesis of N5 (VII)

a) *Benzoylation of H-Acid.* H-Acid (8-hydroxy-1-naphthylamine-3,6-disulphonic acid; 4 mmol) was benzoylated with (5.2 mmol) m-nitrobenzoyl chloride in 7 ml H_2O containing sodium acetate (6.2 mmol). The reaction mixture was stirred for 14 hr at room temperature and evaporated to dryness *in vacuo*. The residue was dissolved in hot 20% ethanol. After 24 hr the yellow precipitate was collected by centrifugation with a yield of 68%.

b) *Reduction of nitrobenzoyl H-acid.* 2.3 mmol of the nitro compound was dissolved in 50 ml of H_2O and reduced over 110 mg of catalyst (5% palladium on carbon) in a Parr bottle at 3 atm at room temperature for 3 hr. The catalyst was removed by filtration and the solution was concentrated by rotary evaporation to 5 ml. Then 10 ml of ethanol was added. The precipitate was collected by centrifugation and recrystalized from 20% ethanol with a yield of 70%.

c) *Phosgenation and purification.* The monosodium salt of the aminobenzoyl H-acid (1.5 mmol) was dissolved in 1 ml of H_2O and 1.5 mmol of Na_2CO_3 was added. The solution was placed in a 3 neck flask and stirred rapidly. A solution of triphosgene (1.5 mmol in 1.5 ml of toluene) and a solution of Na_2CO_3 (9 mmol in 5 ml of H_2O) were added dropwise simultaneously. After 15 hr at room temperature, 5 ml of $CHCl_3$ was added, stirred for 10 min, and the organic layer was removed. The aqueous layer was acidified with 0.1 N HCl to pH = 3.5 and left for 12 hr. The jelly-like precipitate was separated by centrifugation and washed twice with 10 ml of ethanol and dried *in vacuo*. Pure N5 was

prepared by chromatography on Sephadex G-25 on a 25 x 5 cm column with a yield of 50%. Alternatively, the product may be purified by reverse phase liquid chromatography on a C18 stationary phase eluted with a gradient of 0 to 30% acetonitrile in .05% TFA adjusted to pH 2.5 with ammonia. The identity of the product was confirmed by elemental analysis. The ^{1}H NMR spectrum and FAB mass spectrum are consistent with the desired structure (see Figures 16 and 17).

Other suitable chromatographic separation methods include reverse phase HPLC with or without ion pairing reagents or gel permeation chromatography or flash chromatography. Crystallization is also useful in purifying the compounds of the invention when necessary.

The other claimed compounds are prepared using similar chemical procedures and the appropriate starting materials. The appropriate starting materials are identified in the Table wherein amino G-acid is 2-naphthylamine-6,8-disulphonic acid; Freund's acid is 1-naphthylamine-3,6-disulphonic acid; C-acid is 2-naphthylamine-4,8-disulphonic acid; amino J-acid is 2-naphthylamine-5,7-disulphonic acid; H-acid is 8-hydroxy-1-naphthylamine-3,6-disulphonic acid; 2R-acid is 8-hydroxy-2-naphthylamine-3,6-disulphonic acid; and B-acid is 1-naphthylamine-4,6,8-trisulphonic acid.

Hydroxy sulphonated naphthylamines are available from commercial sources or can be directly synthesised. For example, over twenty different disulphonated hydroxynaphthylamine isomers are described in *Beilstein* (see Band 14).

Similar descriptions are readily available in the literature to produce disulphonated naphthylamines, monosulphonated hydroxynaphthylamine

and disulphonated naphthols which can be used directly or modified by known methods of electrophilic substitution to the corresponding hydroxy disulphonated naphthylamines.

Structural Series	Number of bridging rings		
	4	3	2
amino G-acid	CPD10	-	CPD9
Freund's acid	N3	-	N2
C-acid	CPD11	-	N4
amino-J-acid	CPD13	-	CPD12
H-acid	CPD8	N6	N5
2R-acid	CPD15	-	CPD14
B-acid	CPD16	-	N1

For compounds with 4 bridging rings the aminobenzoyl acid produced in step b) undergoes a further round of benzoylation as in step a) before being phosgenated and purified as in step c).

It is also possible to prepare asymmetric compounds using this chemistry by adding two different amino-containing compounds to the phosgenation reaction. This will result in three products which can then be separated by, for example, the chromatography procedures described above.

As an example, phosgenation of m-aminobenzoyl H-acid and m'-aminobenzoyl-m-aminobenzoyl H-acid yields three possible products (N5, N6 and CPD8) which can readily be separated by reversed phase chromatography (see Figure 18). N6 is the three-bridging-ring compound

formed by H-acid (N6 in Figures 1 and 18) and is the same as VIII.

The compound having the shortest retention time on HPLC (Figure 18), is N5. The compound having the longest retention time on HPLC (Figure 18), is CPD8.

The syntheses of various of the suramin analogues and compounds of the invention have been disclosed in Balaban & King (1927) *J. Chem. Soc.* December edition, pages 3068-3097 which is incorporated herein by way of reference.

The syntheses of various of the compounds are now described in detail:

Syntheses of N2 and N3

a) Precursor of N2 and N3 (m-*Nitrobenzoyl*-1-*naphthylamine*-3:6-*disulphonic Acid*) - 1-Naphthylamine-3:6-disulphonic acid (Freund's acid, 12.1 g) was dissolved in 40 cc of *N*-sodium hydroxide (1 mol) and 20 cc of water and treated with 14.85 g (2 mols) of *m*-nitrobenzoyl chloride, all at once, and the mixture was shaken for ½ hour with addition of a few drops of ether. Three successive portions (each 40 cc) of *N*-sodium hydroxide were then added at half-hourly intervals with subsequent shaking. The solution was made distinctly acid to Congo-paper, the *m*-nitrobenzoic acid removed by ether, the aqueous liquor neutralised to litmus and concentrated under reduced pressure at 50°, and two crops of the *disodium* salt collected (yield, 75%). This salt is readily soluble in water (1 in 26) and is precipitated in microscopic, triangular leaflets on addition of sodium chloride. For analysis it was crystallised from 50% alcohol (Found: Loss at 140°, 16.3. $C_{17}H_{10}O_9N_2S_2Na_2,5½H_2O$ requires H_2O, 16.6%. Found in dried salt: Na, 9.1. $C_{17}H_{10}O_9N_2S_2Na_2$ requires

Na, 9.3%). An aqueous solution of the salt is neutral to litmus and gives crystalline precipitates on addition of calcium, magnesium, and barium chlorides. The salt will also crystallise unchanged from solutions which are acid to Congo-paper.

b) Precursor of N2 and N3 (m-*Aminobenzoyl*-1-*naphthylamine*-3:6-*disulphonic Acid*) - To a solution of 25 g of the crude sodium salt (above described) in 195 cc of 2*N*-sodium hydroxide at 0°, ferrous chloride (70 g; 7 mols) in 100 cc of water was added slowly with stirring, followed by 195 cc of 2*N*-sodium hydroxide to make the solution definitely alkaline. After filtration the ferric hydroxide sludge was extracted twice with 250 cc of 0.2*N*-sodium hydroxide each time and the solution was neutralised and concentrated at 50°; the sparingly soluble azoy-compound (1.7 g) separated first, followed by the *disodium* salt (13.5 g) of the required amino-acid. This crystallises from water, in which it is very readily soluble, in fine needles (Found: Loss at 160°, 16.6. $C_{17}H_{12}O_7N_2S_2Na_2,5H_2O$ requires H_2O, 16.2%. Found in anhydrous salt: Na, 9.5. $C_{17}H_{12}O_7N_2S_2Na_2$ requires Na, 9.9%). It gives a moderately easily soluble *diazo*-compound free from chloride but containing sodium, as a primrose yellow precipitate of needles. This couples with glyoxaline derivatives in aqueous sodium carbonate, the solutions having a cherry-red colour. The disodium salt in concentrated solution gives a *calcium* salt, microscopic prisms, and a *barium* salt, sheaves of flat prisms, on addition of saturated calcium or barium chloride. The *silver* salt separates similarly as a microcrystalline powder.

The *sodium hydrogen* salt is much less soluble than the disodium salt and rapidly separated in flattened prisms when the latter (2.9 g) in 5 cc of water was treated with 1 cc of concentrated hydrochloric acid (yield, 2.6 g) (Found: Loss at 160°, 15.6 $C_{17}H_{13}O_7N_2S_2Na,4½H_2O$ requires H_2O.

15.4%. Found in anhydrous salt: Na, 5.1. $C_{17}H_{13}O_7N_2S_2Na$ requires Na, 5.2%).

The *azoxy-compound* is readily soluble in 8 parts of hot water and crystallises in yellow, silky, hair-like needles (Found: Loss at 95°, 18.5. $C_{34}H_{20}O_{15}N_4S_4Na_4,12H_2O$ requires H_2O, 18.6%. Found in anhydrous salt: Na, 9.2. $C_{34}H_{20}O_{15}N_4S_4Na_4$ requires Na 9.7%).

c) Precursor of N3 (m'-*Nitrobenzoyl*-m-*aminobenzoyl*-1-*naphthylamine*-3:6-*disulphonic Acid*) - the disodium salt of the preceding acid (10.7 g, 87% anhydrous salt) dissolved in 50 cc of water was nitrobenzoylated with two equivalents of acid chloride as described in the first stage of the synthesis. The final solution was made distinctly acid to Congo-paper and the gelatinous precipitate was collected and dissolved in water. The two solutions were then thoroughly extracted with ether, combined, neutralised, and concentrated at 50°, and the successive crops of gelatinous nitro-acid collected. These were dissolved in boiling water and treated with 10 volumes of alcohol and the solution was kept until the gelatinous nitro-compound which first separated had changed into a crystalline mass of soft, white needles (yield, 11 g) (Found in product from 90% alcohol: Loss at 160°, 11.0. $C_{24}H_{15}O_{10}N_3S_2Na_2,4H_2O$ requires H_2O, 10.5%. Found in anhydrous salt: Na, 7.6. $C_{24}H_{15}O_{10}N_3S_2Na_2$ requires Na, 7.5%. This *nitro-amide* is readily soluble in water with a neutral reaction and couples only faintly with Pauly's reagent in sodium carbonate solution. It is fairly readily soluble in boiling methyl alcohol, but does not crystallise well from it.

d) Precursor of N3 (m'-*Aminobenzoyl*-m-*aminobenzoyl*-1-*naphthylamine*-3:6-*disulphonic Acid*) - the preceding nitro-acid (10.2 g) was reduced with ferrous chloride exactly as described at a previous stage.

The alkaline filtrates and extracts of the ferric hydroxide were made neutral to Congo-paper; the thick, gelatinous precipitate which first separated soon became wholly crystalline (yield, 10.1 g). The mother-liquors on concentration only deposited a further 0.25 g. This *amino-acid* is a *monosodium* salt and is soluble in 3 volumes of boiling water, separating as a thick felt of small, silky needles on cooling (Found: Loss at 160°, 24.0. $C_{24}H_{18}O_8N_3S_2Na,10H_2O$ requires H_2O, 24.2%. Found in anhydrous salt: Na, 3.0. $C_{24}H_{18}O_8N_3S_2Na$ requires Na, 4.1%). An aqueous solution of the salt colours Congo-paper faintly blue. The salt forms a sparingly soluble *diazo*-compound, crystallising in microscopic needles, which couples with alkaline β-naphthol with the usual red colour. The diazo-compound is free from chloridion but contains sodium.

e) N2 (s-*Carbamide* of m-*Aminobenzoyl*-1-*naphthylamine*-3:6-*disulphonic Acid*) - Five g of the *m*-aminobenzoyl amide were phosgenated as described in the foregoing section. The solid which had separated was collected, dissolved in 50 cc of hot water, and inoculated with the needle form of the carbamide. The required carbamide then separated in balls of long, silky needles (yield, 3.75 g). This *carbamide* separates from concentrated aqueous solutions in gelatinous, rounded masses which are optically anisotropic, showing black crosses under the polarising microscope. On keeping in more dilute solution, they become transformed into the needle form. It is also readily salted out in gelatinous, anisotropic masses (Found: Na, 7.2; loss at 160°, 21.7. $C_{35}H_{22}O_{15}N_4S_4Na_4,14½H_2O$ requires Na; 7.5; H_2O, 21.4%).

f) N3 (s-*Carbamide of* m'-*Aminobenzoyl*-m-*aminobenzoyl*-1-*naphthylamine*-3:6-*disulphonic Acid*) - the phosgenation was carried out in the same way as the preceding. Complete separation of the partly precipitated carbamide was effected by addition of sodium chloride. The

gelatinous solid from 6 g of amino-compound was dissolved in 30 cc of hot water, neutralised, and treated with 7 volumes of alcohol. On keeping, the carbamide separated in finely divided and partly anisotropic particles free from chloride. The product was again crystallised and gave 3.6 g of carbamide (Found: Loss at 160°, 17.0; Na, 6.2. $C_{49}H_{32}O_{17}N_6S_4Na_4,14H_2O$ requires H_2O, 17.4; Na, 6.3%). This *carbamide*, so prepared, is very soluble in water and separates from concentrated solutions in voluminous, anisotropic, waxy masses. It is precipitated by strong mineral acid as a fine, amorphous precipitate, but is only salted out with difficulty and yields a sparingly soluble, amorphous *barium* salt.

Syntheses of CPD9 and CPD10

a) Precursor of CPD9 and CPD10 (m-*Nitrobenzoyl*-2-*naphthylamine*-6:8-*disulphonic Acid*) - G-Acid (9.8 g of anhydrous sodium hydrogen salt) in 120 cc of water was nitrobenzoylated in the usual way. G-Acid is 2-naphthylamine-6:8-disulphonic acid. The solution was diluted with 425 cc of water to dissolve the solid which had separated; it was then acidified and extracted with ether. After neutralisation the solution was concentrated at 50° and the successive crops of gelatinous solid were collected without washing. The combined crops were made up to 150 cc with boiling water, and 150 cc of alcohol added. On keeping, the nitro-amide separated in soft, woolly needles (yield, 14.55 g) (Found: Loss at 160°, 19.9; Na, 7.7 $C_{17}H_{10}O_9N_2S_2Na_2,7H_2O$ requires H_2O, 20.3; Na, 7.4%). This *amide* is readily soluble in hot water and separates on cooling as an anisotropic, gelatinous phase, possibly a semi-rigid liquid crystal line phase, as it flowed when submitted to pressure under a cover slip. Addition of concentrated hydrochloric acid gave an anisotropic gelatinous precipitate, as also did the addition of sodium chloride, which

even in small quantities has a salting-out effect.

b) Precursor CPD9 and CPD10 (m-*Aminobenzoyl*-2-*naphthylamine*-6:8-*disulphonic Acid*) - the foregoing nitro-compound (12.4 g) in 250 cc of water was reduced with ferrous chloride and alkali at 25°. On acidification, the filtrate and alkaline extracts of the ferric hydroxide gave a crystalline precipitate, and the mother-liquors on concentration a further small crop. These were dissolved in 50 cc of boiling water and treated with 20 cc of alcohol. The amide separated on cooling as a compact mass of needles (yield, 8.8 g) (Found: Loss at 160°, 18.2 $C_{17}H_{13}O_7N_2S_2Na,5½H_2O$ requires H_2O, 18.2%. Found in anhydrous solid: Na, 5.1. $C_{17}H_{13}O_7N_2S_2Na$ requires Na, 5.2%). This *amide* is readily soluble in hot water. A hot concentrated solution, when cold, becomes transformed into a viscous liquid crystalline mass with a sheen. This remains unchanged when kept in a stoppered tube for months. The amide forms a sparingly soluble *diazo-compound*, crystallising in needles with a primrose-yellow colour.

c) Precursor of CPD10 (m'-*Nitrobenzoyl*-m-*aminobenzoyl*-2-*naphthylamine*-6:8-*disulphonic Acid*) - the preceding amino-compound (16.3 g) in 370 cc of water was nitrobenzoylated in the usual way. The very viscous solution which resulted was acidified and mixed with an equal volume of alcohol. On keeping at 0°, the precipitate crystallised (yield, 19.5 g). It separated from hot 65% alcohol in minute, soft needles (Found: Loss at 160°, 14.7. $C_{24}H_{15}O_{10}N_3S_2Na_2,6H_2O$ requires H_2O, 14.9%. Found in anhydrous solid: Na, 7.2. $C_{24}H_{15}O_{10}N_3S_2Na_2$ requires Na, 7.5%). This *amide* is soluble in 14 parts of boiling water and separates in anisotropic gelatinous globules. If the amide be left in contact with 18 volumes of water, the original white powder disappears and the whole of the fluid becomes a white elastic mass with a sheen. Under the

polarising microscope it retained the anisotropic sheen and flowed under uneven pressure of the cover-slip. A hot solution treated with concentrated hydrochloric acid deposits a microcrystalline precipitate, possibly of the free disulphonic acid. The amide is very readily salted out and gives sparingly soluble precipitates with calcium, magnesium, and barium chlorides.

d) Precursor of CPD10 (m'-*Aminobenzoyl*-m-*aminobenzoyl*-2-*naphthylamine*-6:8-*disulphonic Acid*) - the preceding nitro-amide (17.4 g) was dissolved in 600 cc of water at 30° and treated with 84 cc of 2*N*-sodium hydroxide. The solution set to a clear anisotropic gel, which gradually disintegrated on addition of 33.6 g of ferrous chloride in 50 cc of water. Finally, a further 84 cc of 2*N*-sodium hydroxide were added, the final reaction being made faintly alkaline to litmus. After being stirred for 1 hour, the solution was warmed to 50° to ensure complete solution and reduction of the nitro-compound. The filtrate and alkaline extracts of the ferric hydroxide on acidification deposited the amino-compound in soft, matted, microscopic needles. Precipitation was completed by addition of sodium chloride. The collected solid was dissolved in the minimum volume (700 cc) of boiling water and treated with 350 cc of alcohol; on cooling, the amino-amide crystallised in clusters of soft, white needles (yield, 14.7 g) (Found: Loss at 160°, 14.5; Na, 3.5. $C_{14}H_{18}O_8N_3S_2Na.5\frac{1}{2}H_2O$ requires H_2O, 15.0; Na, 3.5%). This *amino*-compound is soluble in boiling water; the solution, on cooling, develops a white sheen, due to separation of the liquid crystalline phase. If a small amount of sodium chloride be added to a clear dilute solution, this develops the liquid crystalline sheen, but only temporarily, the amide separating eventually in the solid crystalline condition. Hydrochloric acid also precipitates a liquid crystalline phase, and in very dilute hydrochloric acid a clear solution gives a liquid crystalline *diazo-sulphonate* on addition

of sodium nitrite. The *disodium* salt is readily obtained as well-formed needles by dissolving the sodium hydrogen salt in warm sodium hydrogen carbonate solution and allowing it to cool. The *magnesium, calcium,* and *barium* salts are sparingly soluble in water.

e) CPD9 (s-*Carbamide* of m-*Aminobenzoyl*-2-*naphthylamine*-6:8-*disulphonic Acid* - the parent amino-compound (5 g) was submitted to a treble phosgenation. Separation of the solid, which consisted of very fine, small needles, was completed by saturation with sodium chloride. The product was dissolved in 50 cc of hot water and neutralised. It separated in round, weakly anisotropic nodules, which coalesced on attempted washing. It was redissolved in 25 cc of water, and 25 cc of alcohol added; it then slowly separated in clusters of needles (yield 3.5 g) (Found in substance crystallised from water: Loss at 160°, 19.8. $C_{35}H_{22}O_{15}N_4S_4Na_4,13H_2O$ requires H_2O, 19.6%. Found in anhydrous salt: Na, 10.0. $C_{35}H_{22}O_{15}N_4S_4Na_4$ requires Na, 9.6%). This *carbamide* is very readily salted out. It gives sparingly soluble salts with magnesium, calcium, and barium chlorides. With concentrated hydrochloric acid it gives a precipitate of needles, probably the free tetrasulphonic acid.

f) CPD10 (s-*Carbamide of* m'-*Aminobenzoyl*-m-*aminobenzoyl*-2-*naphthylamine*-6:8-*disulphonic Acid* - the parent amino-compound (5 g) was submitted to a double phosgenation. Separation of the carbamide was completed by addition of sodium chloride. It was then dissolved in 150 cc of boiling water with the aid of sufficient alkali to adjust the reaction to neutrality. To the hot solution 150 cc of alcohol were added and on keeping the carbamide separated. It was again crystallised from 100 cc of water and 325 cc of alcohol (yield, 3.8 g) (Found: Loss at 160°, 19.9. $C_{49}H_{32}O_{17}N_6S_4Na_4,16½H_2O$ requires H_2O, 19.9%. Found in anhydrous salt: Na, 7.6. $C_{49}H_{32}O_{17}N_6S_4Na_4$ requires Na, 7.7%). This *carbamide*

separates from water-alcohol mixtures in weakly anisotropic, gelatinous particles. It is soluble in water; a hot concentrated solution on cooling deposits circular anisotropic clusters, but the surrounding aqueous medium also is anisotropic and flows under pressure of a cover-slip. Crystallisation of the solid phase proceeds at the expense of the liquid crystalline phase. Addition of dilute hydrochloric acid to a solution of the carbamide gives a clear anisotropic gel. The *magnesium*, *calcium*, and *barium* salts are sparingly soluble.

Syntheses of N5 (ie compound VII) and CPD8

a) Precursor of CPD14 and CPD8 (1-m-*Nitrobenzoylamino*-8-*naphthol*-3:6-*disulphonic Acid*) - H-Acid (14.7 g) was suspended in 70 cc of water with addition of 5.5 g of hydrated sodium acetate (1 mol) and nitrobenzoylated as described for earlier members. H-Acid is 8-hydroxy-1-naphthylamine-3:6-disulphonic acid. After acidification to Congo-paper, the heavy crop of crystals was collected, dried, and extracted with ether, leaving 18.2 g of crude amide. The mother-liquors gave a further 2.6 g on concentration. These combined crops were dissolved in boiling water and treated with a quarter of its volume of alcohol. On keeping, the pure amide separated in compact clusters of needles (Found: Loss at 120°, 19.3. $C_{17}H_{10}O_{10}N_2S_2Na_2,7H_2O$ requires H_2O, 19.7%. Found in anhydrous salt: Na, 8.9. $C_{17}H_{10}O_{10}N_2S_2Na_2$ requires Na, 9.0%). This *amide* is readily soluble in warm water and is not precipitated by concentrated hydrochloric acid. It is only salted out with difficulty and separates in a crystalline state. The *barium* salt, tufts of needles, is readily soluble in warm water but much less soluble in cold.

b) Precursor of CPD14 and CPD8 (1-m-*Aminobenzoylamino*-8-*naphthol*-3:6-*disulphonic Acid* - the foregoing nitro-amide (12.8 g) was

reduced with ferrours chloride and alkali at 0° in the usual way. The filtrate and alkaline extracts of the ferric hydroxide on neutralisation gave a copious crystalline precipitate, which was collected, dissolved in 200 cc of boiling water, and treated with 100 cc of alcohol. After an interval, the pure amino-compound separated in delicate needles (yield, 9.3 g) (Found: Loss at 160°, 17.4. $C_{17}H_{13}O_8N_2S_2Na,5H_2O$ requires H_2O, 16.4%. Found in dried salt: Na, 4.7. $C_{17}H_{13}O_8N_2S_2Na$ requires Na, 5.0%). This *sodium hydrogen* salt is not very soluble in warm water and it separates in anisotropic gelatinous particles. A dilute aqueous solution gives an immediate anisotropic precipitate on addition of dilute hydrochloric acid, probably of the disulphonic acid, which separates in microscopic leaflets if the solution be warmed and allowed to cool. The *calcium, magnesium*, and *barium* salts are sparingly soluble, gelatinous precipitates. The *disodium* salt is very soluble in water and crystallises in needles.

c) Precursor of CPD8 (1-m'-*Nitrobenzoyl*-m-*aminobenzoylamino*-8-*naphthol*-3:6-*disulphonic Acid*) - the foregoing amino-compound (10.7 g) was nitro-benzoylated as described at the previous stage. The resulting solution consisted of a viscous syrup containing anisotropic stringy or worm-like masses in suspension. It was diluted with 2000 cc of water, acidified, and extracted with ether. The solution was neutralised and then set to a clear anisotropic gel. It liquefied on rise of temperature and was concentrated at 50° to one-half of its bulk and acidified with 200 cc of concentrated hydrochloric acid. This caused the precipitation of anisotropic liquid threads from which the mother-liquour could be decanted. When these were dissolved in hot water (140 cc) and treated with 280 cc of alcohol, the required salt separated in a filterable condition (yield, 11.8 g) (Found: Loss at 160°, 17.4. $C_{24}H_{15}O_{11}N_3S_2Na_2,7½H_2O$ requires H_2O, 17.6%. Found in dried salt: Na, 7.3. $C_{24}H_{15}O_{11}N_3S_2Na_2$ requires Na, 7.3%). This *disodium* salt is soluble in boiling water and.

on cooling, separates in anisotropic, worm-like growths. A dilute aqueous solution treated with a little sodium chloride sets to a clear anisotropic gel. Under the most favourable conditions for crystallisation - a little water and excess of alcohol - it separates in anisotropic, gelatinous aggregates devoid of recognisable structure.

d) Precursor of CPD8 (1-m'-*Aminobenzoyl*-m-*aminobenzoylamino*-8-*naphthol*-3:6-*disulphonic Acid*) - the preceding nitro-compound (9.0 g) was reduced at 0° with ferrous chloride and alkali. The filtrate and alkaline extracts of the ferric hydroxide were neutralised to Congo-paper and on being kept for 24 hours deposited almost the whole of the amino-compound as an amorphous precipitate. This was dissolved in 100 cc of boiling water and treated with 50 cc of alcohol, it then separated slowly in the amorphous condition (Found: Loss at 160°, 16.9. $C_{24}H_{18}O_9N_3S_2Na,6½H_2O$ requires H_2O, 16.8%. Found in anhydrous salt: Na, 4.3. $C_{24}H_{18}O_9N_3S_2Na$ requires Na, 4.0%). This *sodium hydrogen* salt is not very soluble in boiling water. It separates on cooling in microscopic spicules or leaflets. Its dilute aqueous solution is precipitated by concentrated hydrochloric acid as a gelatinous, anisotropic product, which dissolves on warming and then crystallises in fine needles. It gives an orange colour on diazotisation.

e) N5 ie compound VII (s-*Carbamide of* 1-m-*Aminobenzoylamino*-8-*naphthol*-3:6-*disulphonic Acid*) - Five g of the aminobenzoylamide were submitted to a double phosgenation as described in previous instances. The precipitated solid was collected, dissolved in 15 cc of boiling water, neutralised, and treated with 7 cc of alcohol. The carbamide separated in clusters of gelatinous leaflets (yield, 3.0 g) (Found: Loss at 160°, 19.8. $C_{35}H_{22}O_{17}N_4S_4Na_4,13½H_2O$ requires H_2O, 19.7%. Found in anhydrous salt: Na, 9.2. $C_{35}H_{22}O_{17}N_4S_4Na_4$ requires Na, 9.3%). This *carbamide*

is soluble in boiling water and crystallises, on cooling, in the same way as from dilute alcohol. A solution in dilute nitric acid gives an orange-red solution on boiling. With magnesium, calcium, and barium chlorides it yields sparingly soluble, gelatinous precipitates.

f) CPD8 (s-*Carbamide of* 1-m'-*Aminobenzoyl*-m-*aminobenzoylamino*-8-*naphthol*-3:6-6-*disulphonic Acid*) - Five g of the amino-compound were phosgenated in the usual way. The solution was saturated with sodium chloride, and the solid collected. It was dissolved in 260 cc of boiling water, neutralised, and treated with 200 cc of alcohol. The carbamide separated in gelatinous particles which were isotropic (yield, 3.3 g). (Found: Loss at 160°, 20.6; Na, 6.0. $C_{49}H_{32}O_{19}N_6S_4Na_4,17½H_2O$ requires H_2O, 20.4; Na, 6.0%). This *carbamide* is not very soluble in water. It is readily salted out and gives sparingly soluble salts with magnesium, barium, and calcium chlorides.

Synthesis of N4 and CPD11 (ie compound IV)

a) Precursor of N4 and CPD11 (m-*Nitrobenzoyl*-2-*naphthylamine*-4:8-*disulphonic Acid*) 2-Naphthylamine-4:8-disulphonic acid (C-Acid (12.0 g = 9.7 g of anhydrous monosodium salt)) in 210 cc of water was nitrobenzoylated in the usual way. The solid which had separated was collected, dissolved in the minimum volume of water (900 cc), acidified, and extracted with ether. On neutralisation to litmus, the nitro-amide (12.8 g) separated in a crystalline condition. The mother-liquors and the original mother-liquors of the benzoylation gave a further 2.5 g on concentration. The solid crystallised from 9 volumes of boiling water in clusters of soft, pale yellow needles (Found: Loss at 160°, 15.2; Na, 7.6. $C_{17}H_{10}O_9N_2S_2Na_2,5H_2O$ requires H_2O, 15.4; Na, 7.8%). The most striking property of the salt is the ease with which it is salted out from

aqueous solutions. Such solutions are neutral to litmus and give no precipitate with hydrochloric acid.

b) Precursor of N4 and CPD11 (m-*Aminobenzoyl*-2-*naphthylamine*-4:8-*disulphonic Acid*) - The preceding nitro-amide (equivalent to 9.9 g of anhydrous salt) was dissolved in 275 cc of water and reduced with ferrous chloride and alkali at room temperature. The alkaline extracts of the ferric hydroxide on neutralisation to Congo-paper deposited the main bulk of the required amino-acid, and the mother-liquors on concentration gave a further small quantity (yield, practically quantitative). It crystallised from the minimum volume of boiling water as a voluminous mass of soft, silky needles (Found: Loss at 160°, 18.6; Na, 4.3. $C_{17}H_{13}O_7N_2S_2Na,5½H_2O$ requires H_2O, 18.2; Na, 4.2%). This *sodium hydrogen* salt is sparingly soluble in cold water, yielding a solution neutral to litmus. In dilute hydrochloric acid, it gives a sparingly soluble *diazosulphonate*, which separates in anisotropic, filamentous growths. The *disodium* salt was obtained by dissolving the hydrogen salt in a little water with the aid of sufficient sodium hydrogen carbonate to make the reaction faintly alkaline to litmus. It crystallised in fine needles, but it also occurs in plates. It is soluble in 10 volumes of water at room temperature (Found: Loss at 160°, 22.8. $C_{17}H_{12}O_7N_2S_2Na_2,7½H_2O$ requires H_2O, 22.5%. Found in anhydrous salt: Na, 9.7. $C_{17}H_{12}O_7N_2S_2Na_2$ requires Na, 9.9%).

c) Precursor of N4 and CPD11 (m'-*Nitrobenzoyl*-m-*aminobenzoyl*-2-*naphthylamine*-4:8-*disulphonic Acid*) - The foregoing acid (10.2 g) in 320 cc of water was nitrobenzoylated in the usual way. The final solution was clear, very viscous, and anisotropic. It was diluted with 400 cc of water, acidified to Congo-paper, and extracted with ether. It was then neutralised to litmus, which restored the anisotropic gel condition. If the

solution was more dilute, a gel was not formed, but anisotropic worm- or thread-like growths separated in quantity. These showed the phenomenon of black crosses under the polarising microscope, especially at the ends of growth. In either case, the solution was heated in the boiling water-bath and saturated with sodium chloride with stirring. When cold, the precipitated gelatinous solid was collected in quantitative yield, without washing. 5 G of the air-dried material were dissolved in 75 cc of hot water and treated with 20 cc of concentrated hydrochloric acid. The solution rapidly deposited a thick felt of needles. These were filtered off, dissolved in 25 cc of hot water, and heated with 10 cc of concentrated hydrochloric acid. Fine, ling needles rapidly separated, which were filtered off as dry as possible, rubbed on porous plate, and dried in a vacuum over sulphuric acid and sodium hydroxide. In this way, 2.35 g of almost pure, anhydrous *disulphonic acid* were obtained free from chloride (Found: Na, 0.4%. 0.249 G was equivalent to 8.2 cc of N/10-sodium hydroxide, agreeing with the value required for a mixture of 95% of disulphonic acid and 5% of disodium salt). A repetition of the procedure would probably remove the last traces of sodium. The disulphonic acid, treated with a little water, swells up, forming a clear, viscous, anisotropic fluid in equilibrium with the surrounding aqueous solution. More dilute solutions are clear and set to an anisotropic gel on addition of silver nitrate. If the crude crystalline disulphonic acid obtained after one treatment of the crude disodium salt with hydrochloric acid be dissolved in a small volume of hot water and treated with an equal volume of alcohol, the neutral *disodium* salt separates in soft balls of extremely minute needles (Found: Loss at 160°, 12.7. $C_{24}H_{15}O_{10}N_3S_2Na_2,5H_2O$ requires H_2O, 12.8%. Found in anhydrous salt: Na, 7.4. $C_{24}H_{15}O_{10}N_3S_2Na_2$ requires Na, 7.5%). The mother-liquors, however, are strongly acid to Congo-paper. This salt may be obtained directly from the original crude disodium salt by dissolving it in hot water and adding

alcohol, but the process through the disulphonic acid is preferable. The disodium salt is moderately easily soluble in cold water and on addition of a little sodium chloride the solution sets to a clear anisotropic gel, a state also obtained by dissolving the disodium salt in warm water and allowing the solution to cool. This gel remains unchanged in a sealed vessel for many months. The *barium* and *calcium* salts are very sparingly soluble, the former crystallising in hairs. The *magnesium* salt crystallises in clusters of needles moderately easily soluble in water.

d) Precursor of CPD11 (m'-*Aminobenzoyl*-m-*aminobenzoyl*-2-*naphthylamine*-4:8-*disulphonic Acid*) - The foregoing disodium salt (7.6 g) in 250 cc of water was reduced with ferrous chloride and alkali at 25°. The filtrate and alkaline extracts of the ferric hydroxide were made faintly acid to Congo-paper; the amino-compound then separated as a voluminous, anisotropic, gelatinous product which, on filtration, was left as a white, soapy solid. It was dissolved in 350 cc of boiling water and treated with an equal volume of alcohol; it then separated slowly in gelatinous, oat-shaped, anisotropic crystals (yield, 6.15 g) (Found: Loss at 160°, 17.1; Na, 3.3. $C_{24}H_{18}O_8N_3S_2Na,6½H_2O$ requires H_2), 17.2; Na, 3.4%). This *sodium hydrogen* salt is sparingly soluble in hot water, and a boiling saturated aqueous solution when allowed to cool gives two phases, a clear supernatant liquor and anisotropic aggregates simulating ill-formed, crystalline growths. On filtration on hardened paper under pressure, both phases passed through, yielding a macroscopically homogeneous filtrate with a crystalline sheen. Examined microscopically, it showed complete absence of particles, the whole fluid being anisotropic under polarised light. On prolonged centrifuging there is a separation of a clear aqueous upper layer and a more uniform orientation of the sheen in spiral form in the lower layer.

e) N4 (s-*Carbamide of* m-*Aminobenzoyl-2-naphthylamine*-4:8-*disulphonic Acid*) - For the preparation of this carbamide, 80 volumes of water were required and a double phosgenation was necessary. At the end of the phosgenation the crude carbamide separated in the liquid crystalline state, in the form of stringy, anisotropic masses. After saturation with sodium chloride, the product could be filtered off on hardened paper and after neutralisation with alkali it was dissolved in 9 volumes of hot water and treated with an equal volume of alcohol. The carbamide separated on keeping in clusters of small needles. The yield was 10.5 g from 10.0 g of initial material (Found: Loss at 160°, 18.4; Na, 7.5. $C_{35}H_{22}O_{15}N_4S_4Na_4,12H_2O$ requires H_2O, 18.4; Na, 7.8%). This *carbamide* is readily soluble water, is fairly readily salted out, and is precipitated by excess of concentrated hydrochloric acid in the liquid crystalline state; if, however, the solution be warmed and allowed to cool slowly, the carbamide separates in discrete crystalline growths. The *calcium* and *barium* salts are sparingly soluble, the latter notably so.

f) CPD11 (s-*Carbamide* of m'-*Aminobenzoyl*-m-*aminobenzoyl*-2-*naphthylamine*-4:8-*disulphonic Acid*) - The parent amine (5 g) was phosgenated in the usual way, and precipitation of the crude product completed by addition of sodium chloride. It was dissolved in 30 cc of boiling water, neutralised, and treated with 70 cc of alcohol. On keeping, the carbamide separated without definite recognisable structure, but the product was anisotropic (yield, 4.7 g) (Found: Loss at 160°, 18.2; Na, 5.7, 6.1. $C_{49}H_{32}O_{17}N_6S_4Na_4,14½H_2O$ requires H_2O, 17.9; Na, 6.3%). this *carbamide* is soluble in water and crystallises from concentrated solutions in microscopic, circular, crystalline aggregates. It is readily precipitated, possibly as the free tetrasulphonic acid, by addition of concentrated hydrochloric acid in a similar crystalline form. The *magnesium, barium.* and *calcium* salts are sparingly soluble.

Syntheses of CPD12 (ie compound V) and CPD13

a) Precursor of CPD12 and CPD13 (m-*Nitrobenzoyl*-2-*naphthylamine*-5:7-*disulphonic Acid*) 2-Naphthylamine-5:7-disulphonic acid - (Amino-J-acid (10.2 g = 9.7 g of anhydrous salt)) in 100 cc of water was nitrobenzoylated as described above. After acidification, extraction with ether, and neutralisation the solution was concentrated at 50° and the successive crops were collected without washing, until sodium chloride began to separate. The crude solid was dissolved in 50 cc of hot water and treated with 50 cc of alcohol; it then gradually separated in clusters of small needles (yield, 15.2 g) (Found: Loss at 160°, 16.9; Na, 7.4. $C_{17}H_{10}O_9N_2S_2Na_2,5½H_2O$ requires H_2O, 16.6; Na, 7.7%). This *nitro-amide* is extremely readily soluble in water, and very concentrated aqueous syrupy, solutions deposit fibre- or worm-like anisotropic growths at first, but on keeping, fine needles.

b) Precursor of CPD12 and CPD13 (m-*Aminobenzoyl*-2-*naphthylamine*-5:7-*disulphonic Acid*) - The nitro-amide was reduced at room temperature with ferrous chloride and alkali as described above. The combined alkaline filtrates and extracts of the ferric hydroxide on neutralisation to Congo-paper gave a copious, white, microcrystalline precipitate of the amino compound. The yield was practically quantitative. The solid was dissolved in the minimum volume of boiling water (about 30 volumes). When cold, the product consisted of an opaque, viscous, anisotropic fluid with elastic properties and possessed of a crystalline sheen. There was no separation into two phases on prolonged centrifuging. The liquid crystalline condition was unaltered by addition of dilute acids, but either alcohol or sodium chloride precipitated anisotropic gelatinous masses. On keeping for various periods, 5 days to 3 months, the viscous fluid passed spontaneously into a more stable

condition with separation of microscopic needles in almost quantitative amount (Found: Loss at 160°, 14.4; Na, 4.6. $C_{17}H_{13}O_7N_2S_2Na,4H_2O$ requires H_2O, 14.0; Na, 4.4%). When diazotised in dilute acid solution, it yields a gelatinous *diazo-compound*. The *disodium* salt is extremely soluble in water, the solution soon becoming brown.

c) Precursor of CPD12 and CPD13 (m'-*Nitrobenzoyl*-m-*aminobenzoyl*-2-*naphthylamine*-5:7-*disulphonic Acid*) - The foregoing amino-compound (10.3 g) in 170 cc of water was nitrobenzoylated as described for previous members. The final solution was neutral and contained the required nitro-amide in suspension as a finely-divided, crystalline solid. This was collected and the mother-liquors after removal of nitrobenzoic acid and concentration gave only a small quantity of nitro-amide. The combined solids crystallised from 150 cc of boiling water, with final addition of 150 cc of alcohol, in small, silky needles (yield, 14.0 g) (Found: Loss at 160°, 21.1; Na, 5.6. $C_{24}H_{15}O_{10}N_3S_2Na_2,9H_2O$ requires H_2O, 20.9; Na, 5.9%). This *amide* is readily soluble in warm water and separates from the solution, on cooling, as a white, anisotropic, gelatinous mass. From very dilute solutions it separates in microscopic needles. Its aqueous solution is neutral to litmus, and sodium chloride produces an anisotropic, gelatinous precipitate.

d) Precursor of CPD13 (m'-*Aminobenzoyl*-m-*aminobenzoyl*-2-*naphthylamine*-5:7-*disulphonic Acid*) - The foregoing nitro-compound (7.7 g) in 250 cc of water was reduced at 30-35° with ferrous chloride and alkali. The faintly alkaline mixture was stirred for 2 hours; all traces of the white, gelatinous ferrous salt had then disappeared. The filtrate and alkaline extracts of the ferric hydroxide on neutralisation to Congo-paper gave a gelatinous, anisotropic precipitate of the amino-compound. The mother-liquors gave a further small crop of 0.65 g. The product was

crystallised from 125 cc of boiling water with addition of 125 cc of alcohol and gave 5.35 g of soft balls of microscopic, silky needles of the *sodium hydrogen* salt (Found: Loss at 160°, 15.3; Na, 3.8. $C_{24}H_{18}O_8N_3S_2Na,5½H_2O$ requires H_2O, 15.0; Na, 3.5%). This sodium salt is readily soluble in boiling water, and on cooling, the solution yields a white, turbid, anisotropic fluid with a crystalline sheen. Clear aqueous solutions give gelatinous precipitates at high dilutions with 3*N*-hydrochloric acid. It diazotises and then couples in the usual way with β-naphthol. The *disodium* salt is readily soluble in cold water and crystallises in soft, woolly needles (Found: Loss at 160°, 17.1; Na, 6.3. $C_{24}H_{17}O_8N_3S_2Na_2,6½H_2O$ requires H_2O, 16.7; Na, 6.5%).

e) CPD12 (ie compound V) (s-*Carbamide of* m-*Aminobenzoyl*-2-*naphthylamine*-5:7-*disulphonic Acid*) - The amino-compound (10 g) was phosgenated twice as described in the preceding paragraph. The partial separation of leaflets was completed by saturation with sodium chloride. The product after neutralisation was twice crystallised from hot water by addition of 3 volumes of alcohol (yield, 9.9 g) (Found: Loss at 160°, 22.1; Na, 7.3. $C_{35}H_{22}O_{15}N_4S_4Na_4,15H_2O$ requires H_2O, 22.0; Na, 7.5%). This *carbamide* is very soluble in water, crystallising therefrom in pointed leaflets, and is precipitated by sodium chloride in microscopic rods or narrow leaflets. Addition of concentrated hydrochloric acid to a warm solution precipitates leaflets, possibly of a free sulphonic acid. The *barium* salt is sparingly soluble and crystallises in leaflets.

f) CPD13 (s-*Carbamide of* m'-*Aminobenzoyl*-m-*aminobenzoyl*-2-*naphthylamine*-5:7-*disulphonic Acid*) - Five g of the amino-compound in 500 cc of water were phosgenated in the usual way. The resultant acid product consisted of an opalescent jelly similar to raw white of egg. It was optically anisotropic and devoid of visible particles. When

neutralised, it became definitely biphasic to the eye with separation of the sodium salt. Separation was completed by addition of sodium chloride. The product was collected, dissolved in 120 cc of boiling water, and treated with 120 cc of alcohol. If the alcohol be omitted, the solution sets to a clear anisotropic gel. The product from 50% alcohol was amorphous (yield 3.6 g) (Found: Loss at 160°, 10.3; Na, 6.5. $C_{49}H_{32}O_{17}N_6S_4Na_4,8H_2O$ requires H_2O, 10.7; Na, 6.9%). This *carbamide* is readily precipitated, possibly as the free sulphonic acid, by concentrated hydrochloric acid as a crystalline liquid, but from more dilute solutions, at first as a clear anisotropic gel but later in discrete crystalline growths. If the solution be heated and allowed to cool, it deposits circular tufts of leaflets. The *calcium* and *barium* salts are both sparingly soluble.

Syntheses of CPD14 (ie compound VI) and CPD15

a) Precursor of CPD14 and CPD15 (2-m-*Nitrobenzoylamino*-8-*naphthol*-3:6-*disulphonic Acid*) 8-Hydroxy-2-naphthylamine-3:6-disulphonic acid - (2R-Acid (24.2 g of commercial disodium salt)) was dissolved in 150 cc of water and nitrobenzoylated in the usual way. The highly coloured but clear solution was acidified to Congo-paper and on being kept for 12 hours at 0° gave 37 g of crystalline solid, which, on ether extraction, gave 27.0 g of amide. The original aqueous liquors after extraction with ether and concentration gave a further 2 g of amide. The combined crops crystallised from 100 cc of water in small prisms (yield, 25.2 g) (Found: Loss at 160°, 13.6. $C_{17}H_{10}O_{10}N_2S_2Na_2,4½H_2O$ requires H_2O, 13.7%. Found in anhydrous salt: Na, 8.7. $C_{17}H_{10}O_{10}N_2S_2Na_2$ requires Na, 9.0%). This *disodium* salt is readily soluble in hot water but sparingly soluble in cold. It forms a *barium* salt, sparingly soluble in water, which crystallises in microscopic clusters of needles. This amide is readily salted out: it gives an eosin-like colour in sodium carbonate

solution with Pauly's reagent. When boiled with dilute nitric acid it gives an orange-red solution.

b) Precursor of CPD14 and CPD15 (2-m-*Aminobenzoylamino*-8-*naphthol*-3:6-*disulphonic Acid*) - The above-described nitro-compound (20.8 g) was reduced at 0° with ferrous chloride and alkali in the usual way. The filtrate and alkaline extracts of the ferric hydroxide on neutralisation to Congo-paper deposited 16.8 g of small needles. The mother-liquor on concentration gave a further 2.1 g. The product was recrystallised from 10 volumes of boiling water (Found: Loss at 160°, 13.6. $C_{17}H_{13}O_8N_2S_2Na,4H_2O$ requires H_2O, 13.5%. Found in anhydrous salt: Na, 4.7. $C_{17}H_{13}O_8N_2S_2Na$ requires Na, 5.0%). This *amide* has a faint acid reaction to Congo-paper. It forms a *diazosulphonate*, fine needles, and it gives a sparingly soluble *barium* salt, granular crystals. It is very readily salted out.

c) Precursor of CPD15 (2-m'-*Nitrobenzoyl*-m-*aminobenzoyl-amino*-8-*naphthol*-3:6-*disulphonic Acid*) - The preceding amino-compound (10.6 g) in 100 cc of water was nitrobenzoylated by the usual process. The final product was a clear anisotropic gel. Addition of a little concentrated hydrochloric acid gave a limpid fluid, but excess gave a clear anisotropic gel. This was stirred with sodium chloride; it then coagulated and could be filtered. It was dissolved in 150 cc of boiling water and treated with 400 cc of alcohol. On keeping the nitro-compound separated in the amorphous state, but gradually transformed into clusters of microscopic needles (yield, 11.5 g) (Found in product from 50% alcohol: Loss at 160°, 17.1. $C_{24}H_{15}O_{11}N_3S_2Na_2,7H_2O$ requires H_2O, 16.7%. Found in dried salt: Na, 7.2. $C_{24}H_{15}O_{11}N_3S_2Na_2$ requires Na, 7.3%). This *nitro-compound* is best obtained white and in needles by stirring it with just insufficient boiling water to dissolve it and adding alcohol gradually to

complete its solution and then several volumes of alcohol. The *calcium* and *barium* salts are sparingly soluble.

d) Precursor of CPD15 (2-m'-*Aminobenzoyl*-m-*aminobenzoylamino*-8-*naphthol*-3:6-*disulphonic Acid*) - The foregoing nitro-compound (12.0 g) was dissolved in 222 cc of 0.5*N*-sodium hydroxide and reduced with ferrous chloride and alkali at 15° in the usual way. The filtrate and alkaline extracts of the ferric hydroxide were acidified and separation of the anisotropic solid was completed by saturation with sodium chloride. The gelatinous solid obtained on filtration was dissolved in 100 cc of boiling water and treated with 300 cc of alcohol. On keeping at 0° for some days, a gelatinous, anisotropic mass filled the fluid. This was collected by filtration and washed free from chloride by 90% alcohol. The mother-liquors were concentrated to a small volume and re-treated in a similar way. The total yield of solid, dried in a vacuum, was 9.0 g. (Found: Loss at 160°, 20.6. $C_{24}H_{18}O_9N_3S_2Na,8½H_2O$ requires H_2O, 20.9%. Found in dried salt: Na, 4.3. $C_{24}H_{18}O_9N_3S_2Na$ requires Na, 4.0%). This *amino-compound* is readily soluble in hot water and separates on cooling in gelatinous, anisotropic needles. It is very readily salted out and if such a solution be warmed until clear and be allowed to cool, it sets to a clear anisotropic gel. A dilute solution in hydrochloric acid gives a clear anisotropic, gelatinous solution on addition of sodium nitrite and the product couples with alkaline β-naphthol. Hot dilute nitric acid gives a red coloration, and an alkaline solution couples with diazotised sulphanilic acid with production of an eosin-like coloration.

e) CPD14 (ie compound VI) (s-*Carbamide of* 2-m-*Aminobenzoylamino*-8-*naphthol*-3:6-*disulphonic Acid*) - The parent amine (5.8 g) was submitted to a double phosgenation in the usual way. The precipitated solid was dissolved in 17.5 cc of boiling water with

neutralisation and precipitated by addition of an equal volume of alcohol (yield of material dried in a vacuum, 2.8 g). (Found: Loss at 160°, 13.6. $C_{35}H_{22}O_{17}N_4S_4Na_4,8½H_2O$ requires H_2O, 13.4%. Found in dried solid: Na, 9.2. $C_{35}H_{22}O_{17}N_4S_4Na_4$ requires Na, 9.3%). This *carbamide* is readily soluble in water and crystallises from very concentrated solutions as a microcrystalline powder, but better by addition of sodium chloride. The *calcium* and *barium* salts are sparingly soluble.

The mass spectrum of CPD14 is shown in Figure 11 and the ^{1}H-nmr spectrum of CPD14 shown in Figure 12.

f) CPD15 (*s-Carbamide of* 2-m'-*Aminobenzoyl*-m-*aminobenzoylamino*-8-*naphthol*-3:6-*disulphonic Acid*) - The parent amine (5 g) in 100 volumes of water containing 20 g of sodium carbonate was submitted to a double phosgenation at 30°. The precipitated solid was neutralised, dissolved in 120 cc of boiling water, and treated with 120 cc of absolute alcohol. The yield of material dried in a vacuum over sulphuric acid was 3.0 g. (Found: Loss at 160°, 11.9. $C_{49}H_{32}O_{19}N_6S_4Na_4,9H_2O$ requires H_2O, 11.7%. Found in dried solid: Na, 7.6. $C_{49}H_{32}O_{19}N_6S_4Na_4$ requires Na, 7.5%). This *carbamide* is easily soluble in warm water and is readily salted out as a weakly anisotropic solid. Concentrated hydrochloric acid precipitates a weakly anisotropic, finely divided substance, possibly the free tetrasulphonic acid. The *magnesium*, *calcium*, and *barium* salts are sparingly soluble, gelatinous substances.

Syntheses of N1 and CPD16

a) Precursor of N1 and CPD16 (m-*Nitrobenzoyl*-1-*naphthylamine*-4:6:8-*trisulphonic Acid*) - The parent naphthylaminetrisulphonic acid

(1-naphthylamine-4:6:8-trisulphonic acid) (9.0 g of disodium salt) was nitrobenzoylated in the usual way. After removal of nitrobenzoic acid the neutralised liquors on concentration gave the required amide, which was recrystallised from a small volume of water (yield, 13.55 g) (Found: Loss at 160°, 11.7. $C_{17}H_9O_{12}N_2S_3Na_3,4½H_2O$ requires H_2O, 11.9%. Found in dried substance: Na, 11.4. $C_{17}H_9O_{12}N_2S_3Na_3$ requires Na, 11.5%). This *amide* is very readily soluble in water and is only salted out with difficulty. It is best crystallised from diluted alcohol and then separates in clusters of needles. It forms a readily soluble *calcium* salt and a somewhat less soluble *barium* salt, crystallising in woolly needles.

b) Precursor of N1 and CPD16 (m-*Aminobenzoyl*-1-*naphthylamine*-4:6:8-*trisulphonic Acid*) - 13.3 G of the above nitro-amide were reduced with ferrous chloride and alkali. The neutralised filtrates, freed from iron, were concentrated under reduced pressure at 80° and as much sodium chloride removed as possible by evaporation and filtration and precipitation with alcohol. The amino-compound was then precipitated as an oil by excess of alcohol and gradually solidified. The yield of crude material containing some sodium chloride was 11.1 g. It was not obtained sufficiently pure for analysis. It is extremely soluble in water and is not salted out. It is also soluble in hot methyl alcohol. It diazotises and then couples with β-naphthol. From ethyl-alcoholic solutions containing a little water it crystallises in needles or prisms.

c) Precursor of CPD16 (m'-*Nitrobenzoyl*-m-*aminobenzoyl*-1-*naphthylamine*-4:6:8-*trisulphonic Acid*) - 8 G of the above amino-compound were nitro-benzoylated in the usual way. The neutralised liquor on concentration deposited a felted mass of needles, which were collected without washing, dissolved in 10 cc of hot water, and treated with 50 cc of alcohol. When cool, the required nitro-amide had separated

as a felt of very fine needles (yield, 7.4 g). For analysis, it was recrystallised from 90% alcohol (Found: Loss at 160°, 16.7. $C_{24}H_{14}O_{13}N_3S_3Na_3,8H_2O$ requires H_2O, 16.7%. Found in dried substance: Na, 9.4. $C_{24}H_{14}O_{13}N_3S_3Na_3$ requires Na, 9.6%). This *nitro-amide* is very easily soluble in water, but is readily salted out. On addition of calcium or barium chloride the *calcium* or *barium* salt is precipitated as a gelatinous, anisotropic product, but on keeping the barium salt crystallises in tufts of woolly needles.

d) Precursor of CPD16 (m'-*Aminobenzoyl*-m-*aminobenzoyl*-1-*naphthylamine*-4:6:8-*trisulphonic Acid*) - The preceding nitro-compound (7.5 g) was reduced at 0° with ferrous chloride in the usual way. The solution, neutralised to Congo-paper and free from iron, was concentrated to about 60 cc; it then set to a paste of fine, short needles. These were collected without washing, dissolved in 20 cc of hot water, and treated with 25 cc of absolute alcohol. The product separated as an anisotropic, gelatinous mass filling the solution. It was filtered off and well washed with 80% alcohol, but could not thus be freed from chloride. The yield of material dried in a vacuum was 4.5 g. This product is a *disodium* salt and can be salted out from aqueous solutions, but not very readily. For analysis it was dissolved in a small volume of hot water, neutralised to litmus, and treated with several volumes of alcohol. On keeping, the solution deposited balls of woolly needles of the *trisodium* salt (Found in material dried in a vacuum: Loss at 160°, 8.7. $C_{24}H_{16}O_{11}N_3S_3Na3,3½H_2O$ requires H_2O, 8.4%. Found in anhydrous solid: Na, 9.6. $C_{24}H_{16}O_{11}N_3S_3Na_3$ requires Na, 10.0%). The trisodium salt is not salted out and is extremely readily soluble in water. If the reaction of the original liquors from the reduction be neutral to litmus instead of neutral or acid to Congo, no amino-compound separates until almost all the sodium chloride has been removed and the solution

concentrated to a syrup.

e) N1 (s-*Carbamide of* m-*Aminobenzoyl*-1-*naphthylamine*-4:6:8-*trisulphonic Acid*) - The unisolated amino-compound prepared from 10.g of the corresponding nitro-compound was made up to 500 cc with water and treated with 40 g of anhydrous sodium carbonate, and carbonyl chloride passed in until the solution was acid. The neutralised solution was concentrated and as much sodium chloride removed as possible, first by direct crystallisation, then by addition of ethyl alcohol, and finally by addition of methyl alcohol. The liquor was then concentrated to a small volume and several volumes of methyl alcohol were added so long as there was precipitation of sodium chloride; after a few days, below 0°, the required carbamide separated in soft needles (yield, 6.8 g). For analysis, it was recrystallised under the same conditions from 66% methyl alcohol at -5° (Found in material dried in a vacuum: Loss at 160°, 6.1. $C_{35}H_{20}O_{21}N_4S_6Na_6,4H_2O$ requires H_2O, 5.8%. Found in dried solid: Na, 11.4. $C_{35}H_{20}O_{21}N_4S_6Na_6$ requires Na, 11.9%). This *carbamide* is hygroscopic and extremely readily soluble in water or in 98% methyl alcohol and is not salted out.

f) CPD16 (s-*Carbamide of* m'-*Aminobenzoyl*-m-*aminobenzoyl*-1-*naphthylamine*-4:6:8;*trisulphonic Acid*) - The base prepared from the nitro-compound (13.8 g anhydrous) without isolation was phosgenated in 500 cc of water in presence of 50 g of anhydrous sodium carbonate. The carbamide was isolated in the same way as the preceding member. The yield of crystalline solid containing a little chloride was 9.65 g. For analysis, it was dissolved in 10 cc of hot water and treated with 35 cc of 98% methyl alcohol; it then slowly crystallised, below 0°, in clusters of white needles. It was washed with 80% methyl alcohol, cooled to -5° (yield, 8.1 g). As it was hygroscopic, it was dried in a vacuum (Found:

Loss at 160°, 9.3. $C_{49}H_{30}O_{23}N_6S_6Na_6,8H_2O$ requires H_2O, 9.3%. Found in dried solid: Na, 9.5. $C_{49}H_{30}O_{23}N_6S_6Na_6$ requires Na, 9,8%). This *carbamide* is very soluble in water or methyl alcohol and is not salted out. With saturated barium chloride solution, it gives after some delay a *barium* salt in anisotropic particles. It gives no precipitate with lead acetate, but is precipitated by basic lead acetate.

Thus, the naphthylamine derivatives are converted into their *m*-nitrobenzoyl derivatives and these in turn into the *m*-amino-benzoyl derivatives. The latter are phosgenated to form the *s*-carbamides and have also been converted into the *m'*-nitro-benzoyl-*m*-aminobenzoyl derivatives. The latter have finally been reduced to the *m'*-aminobenzoyl-*m*-aminobenzoyl derivatives, which on phosgenation have given the *s*-carbamides. The general scheme of synthesis is shown below:-

$NH_2R \rightarrow RNH.CO.NHR$

$NO_2.Bz.NHR$ (*First carbamide*)

(1st)$NH_2.Bz.NHR \rightarrow RNH.Bz.NH.CO.NH.Bz.NHR$

$NO_2.Bz.NH.Bz.NHR$ (*Second carbamide*)

(md)$NH_2.Bz.NHBz.NHR \rightarrow RNH.BzNH.BzNH.CO.NHBz.NHBz.NHR$

(*Third carbamide*)

(Bz=$.C_6H_4.CO.$)

Synthesis of bis(8-hydroxy-3,6-naphthalene disulphonic acid) compounds with an alkyl linker (for example IX (≡N7))

The synthesis of a bis-hydroxydisulphonated compound with an alkyl linker may be accomplished according to the method of Mohan *et al* (1993) *J. Medicinal Chemistry* **36(14)**, 1996-2003 incorporated herein by reference. Briefly, 1 mmole (0.341 gram) of 1-amino-8-hydroxy-3,6-

naphthalene disulphonic acid disodium salt is added to 1 ml of dry pyridine and heated to 130°. To this is then added 0.6 mmol (0.11 ml) of suberoyl chloride (suberic acid dichloride) and the mixture is refluxed for 2 hours. The resulting mixture is stirred with 20 ml of heptane and filtered. The residue is suspended in 10 ml of methanol and filtered. This filtrate is suspended in 50 ml ether. The insoluble material is collected by filtration and dissolved in 5 ml of water. The desired product (IX or N7) is purified on a gel filtration column by elution with water. Acetone addition to the fractions containing the desired product results in the precipitation of a light brown powder (0.342 gram, 42% yield) as the tetrasodium salt. This compound contains a six carbon alkyl linker provided by the suberoyl chloride. Linkers of longer length can be made by, for example, substituting octanedioyl dichloride (for an eight carbon linker) or pentanediolyl dichloride (for a ten carbon linker).

Synthesis of bis(8-hydroxy-3,6-naphthalene disulphonic acid) compounds with a phenylsulphonylamino linker (for example X (≡N8))

The synthesis of a bis-hydroxydisulphonated compound with a phenylsulphonylamino linker may be accomplished according to the method of Mohan *et al* (1993) *J. Medicinal Chemistry* **36(14)**, 1996-2003. Briefly, 2 mmole (0.682 gram) of 1-amino-8-hydroxy-3,6-naphthalene disulphonic acid disodium salt is combined with 1.4 mmol (0.490 grams) of 4,4′-biphenyldisulphonyl chloride and 4 mmol (0.336 gram) of $NaHCO_3$ in 20 ml of deionized water at 45° for 10 hours. An additional 0.2 mmol of 4,4′-biphenyldisulphonyl chloride is added at this time and stirring is continued. After 24 hours, an additional 0.4 ml of 4,4′-biphenyldisulphonyl chloride is added and applied to a gel filtration column and eluted with deionized water. Evaporation of the pure

fractions yielded a pale brown powder isolated as the tetrasodium salt (0.087 gram, 5% yield) (X or N7). As will be appreciated by those skilled in the art, it is straightforward to substitute phenyldisulphonyl chlorides which contain additional, for example four, phenyl rings to provide a compound with a longer linking group.

The compounds of the invention can be purified using the chromatographic procedures described above and the structures confirmed using FAB mass spectroscopy and nmr spectroscopy.

It will be appreciated that a suitable linker for a single step coupling can be any compound with two amino-reactive functional groups (eg acid chloride, alkyl halide, carbodiimide activated carboxylic acid, sulphonyl chloride, active esters etc) separated by at least 5 but no more than 30 chemical bonds. The linker may also contain two different reactive groups or it may be coupled in more than one step. Alternatively the hydroxy sulphonated naphthylamine may be first modified to introduce a new functional group which is reactive or modified to be reactive and then coupled to a second linker to produce the final molecule. Linkers may include aliphatic dicarboxylic acids, polypeptides, derivatives of polyenes and polyols or carbohydrates.

The invention will now be described in detail with reference to the accompanying drawings in which:

Figure 1 shows some preferred compounds of the invention.

Figure 2 shows suramin and some of its analogues which are <u>not</u> compounds of the invention but are shown for comparative purposes.

Figure 3 shows the effect of suramin, some compounds of the invention and other suramin analogues on ^{3}H-methylthymidine uptake by quiescent BACE cells in the presence and absence of 1 ng/ml bFGF. BACE cells were seeded into 96 well plates at 1000 cells/well in 10% FCD/DMEM and left 12 to 14 days to quiesce. They were then treated with analogue, with or without 1 ng/ml bFGF and 0.5 μCi/well of ^{3}H-methylthymidine in fresh 2% FCS/DMEM. Cells were harvested 48 h later (n = 5 $\pm$ SEM). Bold face, + 1 ng/ml bFGF. Hatched, no bFGF.

Figure 4 shows dose response curves for the inhibition of bFGF stimulated BACE cell growth by suramin compared to inhibition by compounds of the invention (A): suramin (○), CPD8(●) and CPD11 (■). (B): suramin (○), CPD9 (■) and CPD10 (●). (C): suramin (○), CPD12 (●) and CPD14 (■). Conditions: BACE cells were seeded into gelatin coated 96 well plates at 6000 cells/well in 0.5% FCS/DMEM and left for 12 days to quiesce. Cells were then treated with 1 ng/ml bFGF in 0.5% FCS/DMEM, 0.5 μCi of ^{3}H-methylthymidine per well and the appropriate concentration of suramin or analogue. Cells were harvested 48 h later. (n = 5 $\pm$ SEM).

Figure 5 shows growth curves for BACE cells treated with suramin, suramin analogues and compounds of the invention in the presence of 1 ng/ml bFGF. Control (●), suramin (○), CPD6 (■) and CPD14 (□). Conditions: BACE cells were seeded at 10,000 cells/well into gelatin coated 6 well plates in 5% FCS/DMEM and left 7 days to quiesce. Cells were then treated with 100 μM suramin or analogue with or without 1 ng/ml bFGF in 5% FCS/DMEM. Cells were treated on days at which duplicates were counted. Points are the average of two wells. The experiment was repeated with similar results.

Figure 6 shows growth curves for BACE cells treated with suramin and compounds of the invention. (A): Also treated with 1 ng/ml bFGF; control (●), suramin (○), CPD8 (■) and CPD11 (□). (B): In the absence of bFGF; control (●), suramin (○), CPD8 (■) and CPD11 (□). Conditions as for Figure 3.

Figure 7 shows the comparison of the inhibitory activity of (A) suramin; (B) CPD11; and (C) pentosan polysulphate on microvascular (BACE) (●) and large vessel (HUVEC) (○) endothelium.

Figure 8 shows the effect of (i) suramin, (ii) CPD14 and (iii) CPD1 on ^{133}xenon clearance from a polyester sponge implanted subcutaneously in the rat. bFGF (100 ng) was injected daily into the sponge. Control = a sponge alone that received no bFGF. Drugs were administered either directly into the sponge daily (A) or given as a single dose on day 1 into the tail vein (B).

Figure 9 shows that CPD14 can inhibit the growth of epithelial cancer cells.

Figure 10 shows a mass spectrum of CPD14.

Figure 11 shows a ^{1}H nmr spectrum of CPD14.

Figure 12 shows weight loss data for KHT tumour bearing site.

Figure 13 shows growth as a function of drug concentration for DU-145 cells (human refractory prostate cancer) treated with suramin and compounds of the invention.

Figure 14 shows growth as a function of drug concentration for DU-145 cells (human refractory prostate cancer) treated with suramin and compounds of the invention.

Figure 15 shows growth as a function of drug concentration for human aortic smooth muscle cells treated with suramin and compounds of the invention.

Figure 16 shows the 100 MHz ^1H NMR spectrum of N5 (VII) in D_2O.

Figure 17 shows the fast atom bombardment mass spectrum (FAB-MS) of N5 (VII).

Figure 18 shows the separation of N5, N6 and CPD8 by reversed phase chromatography.

EXAMPLES

Example 1: Materials and methods

Bovine adrenal capillary endothelial (BACE) cells were isolated by clonal selection from cultures of collagenase digests as described for their human counterparts (Fawcett, J. *et al* (1991) *Biochem. Biophys. Res. Commun.* **174**, 903-908). Human umbilical vein endothelial cells (HUVECs) were isolated by collagenase digestion of perfused umbilical veins (Jaffe, E.A. *et al* (1973) *J. Clin. Invest.* **52**, 2745-2756) and used up to the fourth passage. NIH Swiss 3T3 fibroblasts were obtained from the American Type Culture Collection, Bethesda, MD, USA. Suramin is available from Bayer AG. Leverkusen, Germany. Stock solutions of suramin, suramin analogues and compounds of the invention were made up in water. sterile

filtered and stored at -80°C. The purity of the compounds was examined by tlc on Merck Kieselgel 60 F_{254} 0.2 mm pre-coated plates run in $CHCl_3$:MeOH:H_2O 10:10:3. All compounds resolved to a single spot except CPD9 and CPD14 which contained a minor contaminant but were nevertheless >95% pure. Human recombinant bFGF is available from British Bio-Technology Ltd, Cowley Road, Oxford or Genzyme Ltd, West Malling, Kent, UK. Pentosan polysulphate is available from Sigma. ^{3}H-Methylthymidine (2 Ci/mmol) was from Amersham plc, Amersham, UK. Fetal calf serum was from J. Bio, Les Ulis, France. All other tissue culture media were prepared at the ICRF Clare Hall Laboratories, South Mimms, UK using methods known in the art.

^{3}H-methylthymidine uptake assay: Cells were seeded into 96 well tissue culture plates (gelatin coated for endothelial cells but not for fibroblasts) in the presence of the specified concentration of FCS and left to quiesce for the number of days indicated for each experiment described in the Figures. Medium was then replaced with fresh 5 or 10% FCS with or without 1 ng/ml of bFGF and 0.5 μCi of ^{3}H-methylthymidine per well and with or without inhibitor. Cells were harvested 48 h later with an automated Pharmacia Wallac 96 well harvester directly onto filter mats. Filter mats were counted in a Pharmacia flat bed betaplate scintillation counter.

Growth curves: Growth curves were determined by seeding cells into 6-well plates (gelatin coated for endothelial cells but not for fibroblasts) in a percentage of FCS specified in each experiment and allowed to quiesce for the indicated number of days. At day zero cells were treated with inhibitor, with or without 1 ng/ml of bFGF in fresh DMEM/serum. Cells were subsequently treated in the same way every two days at which point duplicates were removed with trypsin and counted in a Coulter counter for construction of growth curves.

Determination of high-affinity binding of ^{125}I-bFGF: High-affinity

binding of bFGF to confluent, quiescent BACE cell monolayers was determined as described for bFGF binding to fibroblasts by Moscatelli and Quarto (1989) *J. Cell Biol.* **109**, 2519-2527. Carrier-free bFGF was iodinated with 'enzo-beads' according to manufacturer's instructions. Enzo-beads are manufactured by and are generally available from Bio-Rad. Total binding was determined with 1 ng/ml of labelled bFGF in 100 ng/ml of unlabelled bFGF. Specific binding was determined by competition with a ten fold excess of cold bFGF (ie 1 μg/ml).

Example 2: Inhibition of Swiss 3T3 fibroblast and capillary endothelial cell ^{3}H-methylthymidine uptake by suramin and compounds of the invention

The original screen of the effects on ^{3}H-methylthymidine uptake in 3T3 fibroblasts and later in BACE cells of the suramin analogues identified several that were at least equipotent if not more effective in blocking bFGF stimulated thymidine uptake as suramin itself (Figures 1 and 3). We chose analogues showing the most potent inhibition of BACE cells and of them, those of low toxicity in mice (see Table II, Example 5), for further study of their effects on capillary endothelial cell growth.

Figure 4a shows dose response curves for the inhibition of BACE cell ^{3}H-methylthymidine uptake by suramin and the analogues CPD8 and CPD11. Figure 4b gives similar data for the analogues CPD9 and CPD10. It is clear from these figures that the analogues CPD8 and CPD11 appear an order of magnitude more potent in the inhibition of bFGF stimulated BACE cell ^{3}H-methylthymidine uptake than is suramin. Similarly, Figure 4c shows that CPD14 had tenfold greater, CPD10 similar and CPD9 10-fold weaker inhibitory activity than suramin.

Example 3: Inhibition of BACE cell growth by suramin, suramin analogues and compounds of the invention

Further studies were conducted on the ability of suramin, suramin analogues and compounds of the invention to inhibit BACE cell growth. Figure 5 shows the effect of two other analogues CPD6 and CPD14 versus suramin on BACE cell growth. Drugs were again tested at a concentration of 100 μM. CPD14 was chosen as another compound which showed strong inhibitory activity in the screen. CPD6 was chosen because although it appeared to have a weak inhibitory activity in the screen, it was known to be at least 1000-fold less toxic than suramin (Table II). The results were quite clear. CPD6 slightly stimulated growth in the presence of bFGF (as was seen with fibroblasts and other analogues of similar structure, Table II), whereas CPD11 was equipotent or slightly more active in blocking bFGF stimulated growth than was suramin. In the absence of bFGF neither suramin or CPD11 showed toxicity up to 9 days when cells were treated with 100 μM drug on alternate days.

Figure 6 shows BACE cell growth curves both in the presence (A) and absence (B) of 1 ng/ml bFGF. Also shown is the effect on stimulated and basal growth of co-addition of 100 μM suramin and of the compounds CPD8 and CPD11. Here inhibition of ^{3}H-methylthymidine uptake by 100 μM drug had shown near maximal inhibition by the analogues CPD8 and CPD11 and substantial (30%) inhibition by suramin. Three points are immediately apparent from Figure 6. (i) All three of suramin, CPD8 and CDP11 elicited substantial inhibition of bFGF stimulated BACE cell proliferation. Suramin held the cell number comparatively static. However, with the CDP8 and CPD11 there was a substantial decrease in cell numbers over a 7 day period which was much more marked with compound CPD8. (ii) In the absence of bFGF (Figure 6b) control growth

was much less but again inhibited by all three of suramin CPD8 and CPD11 relative to controls. However, (iii) in contrast to the long term cytotoxic effect of all three molecules evident by day 9 of treatment of the bFGF treated (proliferating) cells, only CPD8 showed toxicity to the more quiescent cells treated with serum alone (Figure 6b). BACE cells treated in the absence of bFGF with either suramin or CPD11 showed growth over a 9 day period of treatment but much less than of bFGF treated cells. CPD11 appeared to show particularly selective toxicity for proliferating (bFGF treated) as opposed to quiescent (serum) treated capillary endothelium.

The compounds of the invention (some of which are indicated in Figure 1) are effective in inhibiting the bFGF-stimulated growth of Swiss 3T3 cells and bovine adrenal capillary endothelial (BACE) cells, *in vitro*, and may be effective in preventing angiogenesis and tumour vascularisation, *in vivo*.

Compounds of the invention (Figure 1) have been found to be inhibitory for both Swiss 3T3 fibroblasts and BACE cells. Many of these analogues have either two or four bridging amide aromatic rings. It appears that the spaced naphthyl ring structure is essential for inhibitory activity. Some of these compounds (CPD8, CPD10, CPD11 and CPD14) are at least as active, if not 5- to 10-fold more so, as suramin, but are nevertheless significantly less toxic than suramin *in vivo* (Table II). Indeed some compounds, for example CPD9 and CPD12, are 5- to 10-fold less toxic than suramin *in vivo*.

Suramin and CPD14 potently blocked bFGF stimulated growth while CPD6, consistent with its simple naphthyl substituted urea structure (Figure 2) was inactive. Thus, it appears that the analogues that inhibit

^{3}H-methylthymidine uptake by BACE cells also inhibit cell growth.

CPD14 and CPD15 (as shown in Figure 1) together with CPD7 (as shown in Figure 2) form a structural series which differs only in the number of intervening rings between the substituted naphthyl rings. Thus, CPD7 is inactive on BACE cells and stimulates Swiss 3T3 cells in the presence of bFGF, whereas CPD14 and CPD15 strongly inhibited both cell types.

Example 4: Therapeutic index of selected compounds.

The therapeutic advantage over suramin of a compound of the invention can be judged using the "therapeutic index".

By "therapeutic index" (TI) we mean:

$$\frac{IC_{50} \text{ of suramin on endothelial cells}}{IC_{50} \text{ of compound on endothelial cells}} \times \frac{\text{MTD of compound}}{\text{MTD of suramin}}$$

By "IC_{50}" we mean the concentration of the said molecule required to inhibit endothelial cell growth by 50%. The concentration may be stated in millimolar units. By "MTD" we mean maximum tolerated dose of the said molecule when administered *in vivo* to mice. The dose may be stated as mg of molecule/20 g of mouse. Endothelial cells are used in determining the IC_{50} value, and hence the T.I. It is preferable to use microvascular endothelial cells, for example BACE cells, but large vessel endothelial cells, such as HUVEC's, or any other endothelial cells, may be useful in determining the T.I. Of course, the greater the therapeutic index is above 1, the greater the therapeutic advantage of the compound over suramin.

The therapeutic indices of some of the compounds of the invention are given in Table I. The data for *in vivo* toxicity in mice are shown in Table II in the next Example.

Table I.

Compound	T.I.
CPD8	26.2
CPD9	0.45
CPD10	1.7
CPD11	26.8
CPD12	86.7
CPD14	83.1

IC_{50} values were obtained from the data disclosed in the Examples and in Figure 4 using BACE cells.

Example 5: Mouse toxicity data for suramin analogues and compounds of the invention that inhibit bFGF driven BACE cell proliferation.

Table II

Suramin Analogue	Maximum Tolerated Dose (mg/20g)	nmols/20g
suramin	2	1.4
CPD8	5	4.39
CPD9	10	11.5
CPD11	5	4.5
CPD10	5	4.5
CPD12	10	12.95
CPD13	5	4.95
CPD14	5	5.54
CPD15	2	1.75
CPD16	2	1.57
N5	5	5.54

Drug dosage was increased in the following increments 2, 5, 10 and 20 mg per 20 g of mouse body weight. The maximum tolerated dose is the maximum dose within these increments that could be administered without death.

Example 6: Comparison of the inhibition of ^{3}H-methylthymidine uptake in BACE cells and HUVECs by pentosan polysulphate, suramin and CPD11

It has been disclosed that pentosan polysulphate (PPS) inhibits K-FGF (FGF-4) stimulated endothelial proliferation (Wellstein, A. *et al* (1991) *J.*

Natl. Cancer Inst. **83**, 716-720). Figure 7 compares the inhibition of ^{3}H-methylthymidine uptake by (A) suramin, (B) CPD11 and (C) PPS in both HUVEC and BACE cells. Inhibition of ^{3}H-methylthymidine uptake by capillary cells occurred at a lower concentration of drug than was required to block bFGF stimulated uptake by HUVECs. Indeed with HUVECs in no case was significant inhibition observed with less than millimolar concentrations of inhibitor whereas suramin and CPD11 gave nearly 50% inhibition at 1 μM in BACE cells. We conclude that microvascular endothelium is more sensitive to inhibition by these compounds than is large vessel endothelium.

Example 7: Comparative *in vitro* toxicity

A differential toxic effect on proliferating compared to quiescent endothelium would be useful in the clinic. In comparative *in vitro* toxicity experiments with proliferating versus quiescent endothelium differences were found with suramin, CPD8 and CPD11. In the presence of bFGF all three molecules inhibited growth, but in contrast, in the absence of bFGF slow growth was seen on treatment with CPD11, suramin held the cells cytostatic and CPD8 was cytotoxic over a period of days. That is compound CPD11 appears to be selectively toxic for rapidly proliferating (bFGF treated) capillary endothelium. Similar observations have been reported for tumour necrosis factor-α (TNF-α) on bFGF treated BACE cells (Schweigerer, L. *et al* (1987) *Biochem. Biophys. Res. Commun.* **143**, 997-1004) and probably plays a role in TNF-α induced haemorrhagic tumour necrosis.

Example 8: Comparison of the effect of compounds on microvascular and large vessel endothelial cells

In view of the differential response of large vessel and microvascular endothelium to growth factors (Toi, M. *et al* (1991) *Biochem. Biophys. Res. Commun.* **174**, 1287-1293; McCarthy, S.A. *et al* (1991) *Trends in Pharmacol. Sci.* **12**, 462-467) it is important to study growth inhibitory activity of potential anti-angiogenic compounds with microvascular (eg BACE cells) as opposed to large vessel (HUVEC) endothelium. We compared the inhibitory activity of suramin, CPD11 and PPS on bFGF stimulated large vessel (HUVEC) and capillary (BACE) endothelium. It is clear that while suramin, CPD11 and PPS can block ^{3}H-methylthymidine uptake by HUVECs in response to 1 ng/ml of bFGF, significant inhibition is seen only at millimolar concentrations of inhibitor. In contrast BACE cells were substantially more sensitive to inhibition by all three inhibitors. Thus IC_{50}s for suramin and CPD11 are in the region of 10 nM, that is 10- to 100-fold lower than for HUVECs. PPS inhibits bFGF driven HUVEC and BACE cell ^{3}H-methylthymidine uptake, and that BACE cells are inhibited at lower concentrations than are HUVEC's. However, in contrast to studies on blocking of conditioned media from SW13 cells transfected with K-FGF we found that PPS was no more active in blocking bFGF driven ^{3}H-methylthymidine uptake in either HUVEC or BACE cells than was suramin or CPD11. This may reflect different interactions with members of the FGF family or a difference in experimental conditions eg bFGF concentrations in our assays.

Example 9: Effect of suramin and derivatives on bFGF stimulated angiogenesis *in vivo*

The study of Pesenti *et al* (1992) *Br. J. Cancer* **66**, 367-372 has shown that i.v. suramin is able to block bFGF induced vascularisation of a gelatin sponge implanted sub-cutaneously in rats. We have employed a similar model to examine the anti-angiogenic activity of the related polyanions. A polyester sponge was employed rather than the gelatin sponge of Pesenti *et al* (1992) *Br. J. Cancer* **66**, 367-372 which unlike the gelatin sponge spontaneously vascularises slowly as a result of an inflammatory response.

In all experiments the primary angiogenic stimulus was 100 ng of bFGF in 25 μl of PBS injected daily directly into the sponge. Polyanion antagonism was delivered in two ways. The polyanion was either mixed with the bFGF immediately prior to daily injection into the sponge, or alternatively given as a single dose (in 400 μl of PBS) into the tail vein on the day of sponge implantation. Figure 8 shows that suramin is able to block bFGF driven sponge angiogenesis when administered daily into the sponge at doses of 3 and 10 mg but not when only 1 mg was given. Figure 8(i)(B) shows that a single dose of 40 mg of suramin i.v. substantially reduces bFGF driven sponge angiogenesis for up to 14 days.

The experiments were repeated with two of the polyanions, namely (CPD14 that effectively blocks bFGF activity *in vitro* and CPD1 that was unable to antagonize bFGF *in vitro*. Figure 8(ii) shows that CPD14 is as effective as suramin at blocking bFGF driven angiogenesis both when administered directly into the sponge or given as a single dose i.v. on the day of sponge implantation. In contrast, Figure 8(iii) shows that CPD1 was unable to prevent bFGF stimulated angiogenesis.

Example 10: Growth inhibition of epithelial cells by suramin and CPD14

Cell culture preparation: Cell lines were harvested by trypsination while in exponential growth phase, and seeded in 96 well plates at 5000 cells per well (180 μL RPMI medium, supplemented with 10% FCS and 4 mM glutamine). Cells were allowed to attach for 2 hours prior to drug addition.

Suramin and CPD14 preparation: Stock solutions were prepared fresh using culture media, to a concentration of 10 mM. Solutions were filter sterilised using a 0.42 μM gauge filter and serial diluted. 20 μL aliquots were added to each well to give a final concentration range of 0.01 - 1000 μM. Cells were allowed to grow for 96 hours.

MTT assay:

I) Drug/medium solution was removed, and the wells were washed twice in warm PBS.

II) 200 μL per well of fresh medium was replaced (37°C), and 50 μL MTT solution was added (2 mg/ml). (Suramin inhibits formation of the formazan crystal product.)

III) Plates were incubated for 4 hours at 37°C.

IV) Medium was tipped off and 150 μL DMSO was added to each well, followed by 25 μL glycine buffer (pH 10.5).

V) Plates were shaken for 5 minutes.

VI) Absorbencies were read immediately at 540 nm.

VII) Growth inhibition was expressed as a % of control, following subtraction of the medium blank.

The results of experiments using H226 cells and MCF-7wt cells are shown in Figure 9; CPD14 can inhibit the growth of epithelial cancer cells.

Example 11: Effects on tumour growth and weight loss and toxicity of CDP14 compared to suramin

8-12 week old category IV female C3H/He mice bred at the UK Medical Research Council Rodent Breeding Unit were used for experiments. KHT tumours were mainitained by sequential passage of tumours *in vivo*. Sub-cutaneous tumours were derived by injection of 2 x 10^5 viable cells (obtained by trypsin/DNAase digestion of a maintenance tumour) into the mid-dorsal pelvic region of the back.

Equimolar doses of the anti-angiogenic drugs were administered by the i.p. route on days 1, 5, 9, 13 and 17 following implantation which is similar to the protocol followed by Walz *et al* (1991) *Cancer Res.* **51**, 3585-3589 incorporated herein by reference. Tumours were measured as soon as they became palpable and volumes calculated from the three orthogonal diameters multiplied by $\pi/6$. Measurements were made at least 4 x weekly and body weights recorded at the same time.

Figure 12 gives the percent change in body weight of mice implanted with KHT tumours comprising three groups, controls and those receiving either suramin or CPD14. Mice receiving suramin showed a marked loss in body weight not seen in either controls or those receiving equimolar quantities of CPD14. In these same experiments a significant anti-tumour effect was seen with both suramin and CPD14. KHT tumours in mice receiving no treatment with anti-angiogenic drug take 14.9 $\pm$ 0.3 days from the time of implant to reach a volume of 200 mm^3. In comparison this time is increased to 19.2 $\pm$ 0.7 and 19.9 $\pm$ 0.5 days for suramin and

CPD14 respectively. Normally tumour bearing mice were sacrificed when tumours reached 500 mm^3 or when there were clinical signs of severe drug toxicity. The latter was only apparent in mice treated with suramin. By day 16, those mice receiving suramin showed poor coat condition, lack of alertness in the eyes, oedema around the feet and the base of the ears, some dermatitis/urticaria, bradypnoea and slight photophobia. Organ histology of suramin treated mice showed in the kidney - minor droplet degeneration of tubules, and in the liver a non-degenerative droplet change. No other organs showed gross histological abnormalities, although it should be noted that on sacrifice the bones of the suramin treated mice were extremely brittle. There were no comparable clinical signs of toxicity in the CPD14 treated mice except for the weight loss on the day of sacrifice. No significant difference in weight was observed in the rats employed in the sponge model between either controls, or those receiving either suramin (3 mg day into the sponge or 40 mg single dose i.v.) or any of the polyanions examined.

Legend for Figure 12

Weight loss data for KHT tumour bearing mice. Control (●), suramin treated (○) and CPD14 treated (■). Suramin (60 mg/kg i.p.) and CPD14 (42 mg/kg) were given on days 1, 5, 9, 13 and 17 after implant. The control group comprised thirty four mice. The suramin and CPD14 groups contained eight mice each. Mice received a sub-cutaneous implant of KHT tumour cells on the back at day 0.

Example 12: Effect of suramin and compounds of the invention on the growth of hormone refractory prostate cancer cells

DU-145 cells are derived from a human prostate tumour. They do not require androgen stimulation and thus have become capable of producing their own growth factors. It is believed that these growth factors are functioning in an autocrine fashion to drive the proliferation of the DU-145 cells.

DU-145 cells were plated at 5000 cells/well into 96 well plates and grown in Dulbecco's minimal essential medium containing 5% fetal calf serum. The cells were maintained at 37° under air-5% CO_2. Suramin and compounds of the invention were introduced into fresh medium at the initiation of the study and the proliferation of the cells was assessed by the MTT procedure, conducted as described in Example 13, after 5 days of exposure to the drugs.

As shown in Figures 13 and 14, suramin and the compounds of the invention are able to effectively block the growth of the DU-145 cells. This is consistent with the known efficacy of suramin in the treatment of hormone refractory prostate cancer in humans. Suramin shows an IC_{50} of about 30 μM in this assay. While CPD 12 shows similar activity to suramin, other compounds of the invention, especially CDP 8 and CPD 11, are substantially more active with IC_{50}'s of 1-2 μM.

Example 13: Effect of suramin and compounds of the invention on the growth of serum stimulated smooth muscle cells in culture

Proliferating smooth muscle cells underlie the intimal thickening that occurs in restenosis. The stimulation for proliferation may come from the

exposure of the smooth muscle cells to growth factors present in serum as a result of procedure-induced damage to the normal endothelial barrier. Compounds which can effectively block smooth muscle growth driven by serum may be expected to have utility in the treatment of restenosis.

Human aortic smooth muscle cells (SMC) were plated at 5000 cells/well into 96 well plates and grown in SMC medium (Clonetics Inc) containing 5% fetal calf serum. The cells were maintained at 37° under air-5% CO_2. Suramin and compounds of the invention were introduced into fresh medium at the initiation of the study and the proliferation of the cells was assessed by the MTT procedure, conducted as described in Example 12, after 5 days of exposure to the drugs.

As shown in Figure 15, suramin is able to inhibit the proliferation of the smooth muscle cells with 50% inhibition occurring at a concentration of about 200-300 μM. All compounds of the invention tested showed inhibitory activity against the smooth muscle cells, several of them showing substantially more activity than suramin. For example, CPD 8 and 14 showed 50% growth inhibition at a concentration of about 50 μM while CPD 11 gave 50% inhibition at 80 μM.

Example 14: Some pharmaceutical formulations

The following examples illustrate pharmaceutical formulations according to the invention in which the active ingredient is a compound of any of the above structures.

Example A: Tablet

Active ingredient	100 mg
Lactose	200 mg
Starch	50 mg
Polyvinylpyrrolidone	5 mg
Magnesium stearate	4 mg
	359 mg

Tablets are prepared from the foregoing ingredients by wet granulation followed by compression.

Example B: Ophthalmic Solution

Active ingredient	0.5 g
Sodium chloride, analytical grade	0.9 g
Thiomersal	0.001 g
Purified water to	100 ml
pH adjusted to	7.5

Example C: Tablet Formulations

The following formulations A and B are prepared by wet granulation of the ingredients with a solution of povidone, followed by addition of magnesium stearate and compression.

Formulation A

	mg/tablet	mg/tablet
(a) Active ingredient	250	250
(b) Lactose B.P.	210	26
(c) Povidone B.P.	15	9
(d) Sodium Starch Glycollate	20	12
(e) Magnesium Stearate	5	3
	500	300

Formulation B

	mg/tablet	mg/tablet
(a) Active ingredient	250	250
(b) Lactose	150	-
(c) Avicel PH 101	60	26
(d) Povidone B.P.	15	9
(e) Sodium Starch Glycollate	20	12
(f) Magnesium Stearate	5	3
	500	300

Formulation C

	mg/tablet
Active ingredient	100
Lactose	200
Starch	50
Povidone	5
Magnesium stearate	4
	—
	359

The following formulations, D and E, are prepared by direct compression of the admixed ingredients. The lactose used in formulation E is of the direction compression type.

Formulation D

	mg/capsule
Active Ingredient	250
Pregelatinised Starch NF15	150
	—
	400

Formulation E

	mg/capsule
Active Ingredient	250
Lactose	150
Avicel	100
	—
	500

Formulation F (Controlled Release Formulation)

The formulation is prepared by wet granulation of the ingredients (below) with a solution of povidone followed by the addition of magnesium stearate and compression.

	mg/tablet
(a) Active Ingredient	500
(b) Hydroxypropylmethylcellulose (Methocel K4M Premium)	112
(c) Lactose B.P.	53
(d) Povidone B.P.C.	28
(e) Magnesium Stearate	7
	700

Drug release takes place over a period of about 6-8 hours and was complete after 12 hours.

Example D: Capsule Formulations

Formulation A

A capsule formulation is prepared by admixing the ingredients of Formulation D in Example C above and filling into a two-part hard gelatin capsule. Formulation B (*infra*) is prepared in a similar manner.

Formulation B

	mg/capsule
(a) Active ingredient	250
(b) Lactose B.P.	143
(c) Sodium Starch Glycollate	25
(d) Magnesium Stearate	2
	—
	420

Formulation C

	mg/capsule
(a) Active ingredient	250
(b) Macrogol 4000 BP	350
	—
	600

Capsules are prepared by melting the Macrogol 4000 BP, dispersing the active ingredient in the melt and filling the melt into a two-part hard gelatin capsule.

Formulation D

	mg/capsule
Active ingredient	250
Lecithin	100
Arachis Oil	100
	—
	450

Capsules are prepared by dispersing the active ingredient in the lecithin and arachis oil and filling the dispersion into soft, elastic gelatin capsules.

Formulation E (Controlled Release Capsule)

The following controlled release capsule formulation is prepared by extruding ingredients a, b, and c using an extruder, followed by spheronisation of the extrudate and drying. The dried pellets are then coated with release-controlling membrane (d) and filled into a two-piece, hard gelatin capsule.

	mg/capsule
(a) Active ingredient	250
(b) Microcrystalline Cellulose	125
(c) Lactose BP	125
(d) Ethyl Cellulose	13
	513

Example E: Injectable Formulation

Active ingredient	0.200 g

Sterile, pyrogen free phosphate buffer (pH7.0) to 10 ml

The active ingredient is dissolved in most of the phosphate buffer (35-40°C), then made up to volume and filtered through a sterile micropore filter into a sterile 10 ml amber glass vial (type 1) and sealed with sterile closures and overseals.

Example F: Intramuscular injection

Active ingredient	0.20 g
Benzyl Alcohol	0.10 g
Glucofurol 75	1.45 g
Water for Injection q.s. to	3.00 ml

The active ingredient is dissolved in the glycofurol. The benzyl alcohol is then added and dissolved, and water added to 3 ml. The mixture is then filtered through a sterile micropore filter and sealed in sterile 3 ml glass vials (type 1).

Example G: Syrup Suspension

Active ingredient	0.2500 g
Sorbitol Solution	1.5000 g
Glycerol	2.0000 g
Dispersible Cellulose	0.0750 g
Sodium Benzoate	0.0050 g
Flavour, Peach 17.42.3169	0.0125 ml
Purified Water q.s. to	5.0000 ml

The sodium benzoate is dissolved in a portion of the purified water and the sorbitol solution added. The active ingredient is added and dispersed. In the glycerol is dispersed the thickener (dispersible cellulose). The two dispersions are mixed and made up to the required volume with the purified water. Further thickening is achieved as required by extra shearing of the suspension.

Example H: Suppository

	mg/suppository
Active ingredient (63 μm)*	250
Hard Fat, BP (Witepsol H15 - Dynamit Nobel)	1770
	2020

*The active ingredient is used as a powder wherein at least 90% of the particles are of 63 μm diameter or less.

One fifth of the Witepsol H15 is melted in a steam-jacketed pan at 45°C maximum. The active ingredient is sifted through a 200 μm sieve and added to the molten base with mixing, using a silverson fitted with a cutting head, until a smooth dispersion is achieved. Maintaining the mixture at 45°C, the remaining Witepsol H15 is added to the suspension and stirred to ensure a homogeneous mix. The entire suspension is passed through a 250 μm stainless steel screen and, with continuous stirring, is allowed to cool to 40°C. At a temperature of 38°C to 40°C 2.02 g of the mixture is filled into suitable plastic moulds. The suppositories are allowed to cool to room temperature.

Example I: Pessaries

	mg/pessary
Active ingredient	250
Anhydrate Dextrose	380
Potato Starch	363
Magnesium Stearate	7
	1000

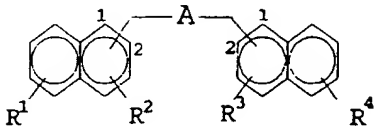
The above ingredients are mixed directly and pessaries prepared by direct compression of the resulting mixture.

Example J: Combined Formulations

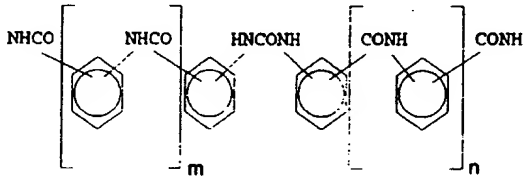
A formulation as defined in any one of Examples A to I wherein the active ingredient comprises an effective amount of the compound as defined in the first aspect of the invention and an effective amount of another female contraceptive agent as defined above; or an effective amount of another cancer therapeutic agent as defined above or an effective amount of an antibiotic or an effective amount of an anti-inflammatory agent or an effective amount of an analgesic or an effective amount of an anaesthetic or an effective combination thereof.

CLAIMS

1. The use of a compound with the structural formula:

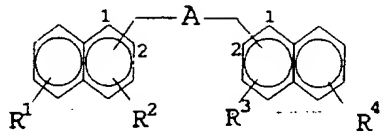
(I) 

wherein R^1 to R^4 are each independently one or more of -X, $-N_3$, $-NO_2$, halo, trifluoromethyl, R^5, OR^5, $-CH_2OR^5$, $-OCOR^5$, $-CH_2OCOR^5$, $-NHCOR^5$, $-CH_2NHCOR^5$, $-NR^5R^6$, $-CH_2NR^5R^6$, $-CH_2NO_2$, $CONR^5R^6$, $CH_2CONR^5R^6$, $-COOR^5$, $-CH_2COOR^5$, -CHO and $-CH_2CHO$ and -X is independently $-SO_3R^5$, $-CH_2PO_3R^5R^6$; $-CH_2SO_3R^5$, $-OSO_3R^5$, $-CH_2OSO_3R^5$, $-CH_2OSO_3R^5$, $-NHSO_3R^5$, $-CH_2NHSO_3R^5$, $-OPO_3R^5R^6$, $-CH_2OPO_3R^5R^6$ and $-PO_3R^5R^6$ where R^5 and R^6 are chosen independently from -H and lower alkyl and wherein A is a chemical group comprising at least 5 and no more than 30 bonds directly linking the naphthyl groups provided that (i) the compound is not suramin and (ii) when A is not

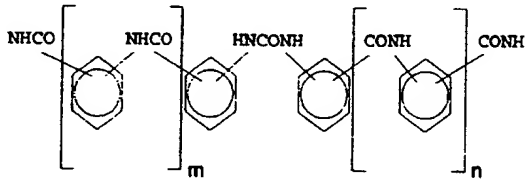


wherein m and n are independently 0, 1 or 2, then at least one of R^1 to R^4 is -OH or an acidic group; or a pharmaceutically acceptable salt, ester, salt of such ester or amide of such compounds, in the manufacture of a medicament for use in treating cancer.

2. The use of a compound with the structural formula:

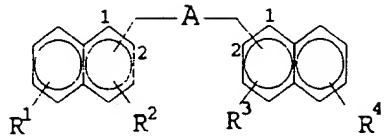
(I) 

wherein R^1 to R^4 are each independently one or more of -X, $-N_3$, $-NO_2$, halo, trifluoromethyl, R^5, OR^5, $-CH_2OR^5$, $-OCOR^5$, $-CH_2OCOR^5$, $-NHCOR^5$, $-CH_2NHCOR^5$, $-NR^5R^6$, $-CH_2NR^5R^6$, $-CH_2NO_2$, $CONR^5R^6$, $CH_2CONR^5R^6$, $-COOR^5$, $-CH_2COOR^5$, -CHO and $-CH_2CHO$ and -X is independently $-SO_3R^5$, $-CH_2PO_3R^5R^6$; $-CH_2SO_3R^5$, $-OSO_3R^5$, $-CH_2OSO_3R^5$, $-CH_2OSO_3R^5$, $-NHSO_3R^5$, $-CH_2NHSO_3R^5$, $-OPO_3R^5R^6$, $-CH_2OPO_3R^5R^6$ and $-PO_3R^5R^6$ where R^5 and R^6 are chosen independently from -H and lower alkyl and wherein A is a chemical group comprising at least 5 and no more than 30 bonds directly linking the naphthyl groups provided that (i) the compound is not suramin and (ii) when A is not

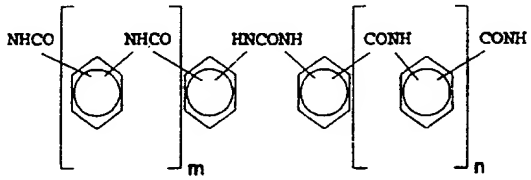


wherein m and n are independently 0, 1 or 2, then at least one of R^1 to R^4 is -OH or an acidic group; or a pharmaceutically acceptable salt, ester, salt of such ester or amide of such compounds, in the manufacture of a medicament for use in reducing undesired angiogenesis.

3. The use of a compound with the structural formula:

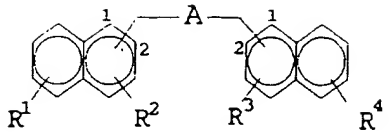
(I) 

wherein R^1 to R^4 are each independently one or more of -X, $-N_3$, $-NO_2$, halo, trifluoromethyl, R^5, OR^5, $-CH_2OR^5$, $-OCOR^5$, $-CH_2OCOR^5$, $-NHCOR^5$, $-CH_2NHCOR^5$, $-NR^5R^6$, $-CH_2NR^5R^6$, $-CH_2NO_2$, $CONR^5R^6$, $CH_2CONR^5R^6$, $-COOR^5$, $-CH_2COOR^5$, -CHO and $-CH_2CHO$ and -X is independently $-SO_3R^5$, $-CH_2PO_3R^5R^6$; $-CH_2SO_3R^5$, $-OSO_3R^5$, $-CH_2OSO_3R^5$, $-CH_2OSO_3R^5$, $-NHSO_3R^5$, $-CH_2NHSO_3R^5$, $-OPO_3R^5R^6$, $-CH_2OPO_3R^5R^6$ and $-PO_3R^5R^6$ where R^5 and R^6 are chosen independently from -H and lower alkyl and wherein A is a chemical group comprising at least 5 and no more than 30 bonds directly linking the naphthyl groups provided that (i) the compound is not suramin and (ii) when A is not

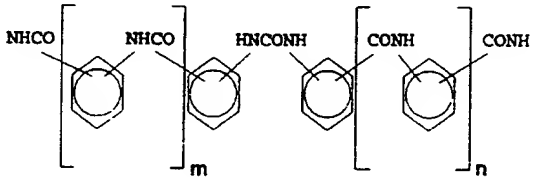


wherein m and n are independently 0, 1 or 2, then at least one of R^1 to R^4 is -OH or an acidic group, or a pharmaceutically acceptable salt, ester, salt of such ester or amide of such compounds, in the manufacture of a medicament for use in treating fibrotic diseases.

4. The use of a compound with the structural formula:

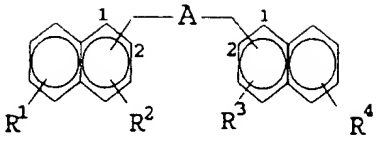
(I) 

wherein R^1 to R^4 are each independently one or more of -X, $-N_3$, $-NO_2$, halo, trifluoromethyl, R^5, OR^5, $-CH_2OR^5$, $-OCOR^5$, $-CH_2OCOR^5$, $-NHCOR^5$, $-CH_2NHCOR^5$, $-NR^5R^6$, $-CH_2NR^5R^6$, $-CH_2NO_2$, $CONR^5R^6$, $CH_2CONR^5R^6$, $-COOR^5$, $-CH_2COOR^5$, -CHO and $-CH_2CHO$ and -X is independently $-SO_3R^5$, $-CH_2PO_3R^5R^6$; $-CH_2SO_3R^5$, $-OSO_3R^5$, $-CH_2OSO_3R^5$, $-CH_2OSO_3R^5$, $-NHSO_3R^5$, $-CH_2NHSO_3R^5$, $-OPO_3R^5R^6$, $-CH_2OPO_3R^5R^6$ and $-PO_3R^5R^6$ where R^5 and R^6 are chosen independently from -H and lower alkyl and wherein A is a chemical group comprising at least 5 and no more than 30 bonds directly linking the naphthyl groups provided that (i) the compound is not suramin and (ii) when A is not

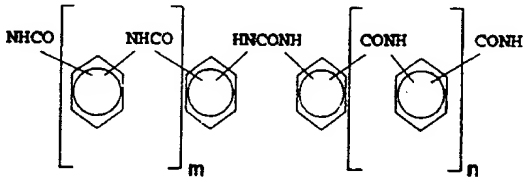


wherein m and n are independently 0, 1 or 2, then at least one of R^1 to R^4 is -OH or an acidic group; or a pharmaceutically acceptable salt, ester, salt of such ester or amide of such compounds, in the manufacture of a medicament for use in treating non-malignant hyper-proliferative diseases.

5. The use of a compound with the structural formula:

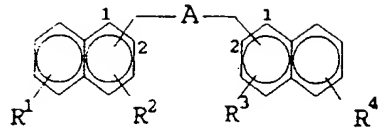
(I) 

wherein R^1 to R^4 are each independently one or more of -X, $-N_3$, $-NO_2$, halo, trifluoromethyl, R^5, OR^5, $-CH_2OR^5$, $-OCOR^5$, $-CH_2OCOR^5$, $-NHCOR^5$, $-CH_2NHCOR^5$, $-NR^5R^6$, $-CH_2NR^5R^6$, $-CH_2NO_2$, $CONR^5R^6$, $CH_2CONR^5R^6$, $-COOR^5$, $-CH_2COOR^5$, -CHO and $-CH_2CHO$ and -X is independently $-SO_3R^5$, $-CH_2PO_3R^5R^6$; $-CH_2SO_3R^5$, $-OSO_3R^5$, $-CH_2OSO_3R^5$, $-CH_2OSO_3R^5$, $-NHSO_3R^5$, $-CH_2NHSO_3R^5$, $-OPO_3R^5R^6$, $-CH_2OPO_3R^5R^6$ and $-PO_3R^5R^6$ where R^5 and R^6 are chosen independently from -H and lower alkyl and wherein A is a chemical group comprising at least 5 and no more than 30 bonds directly linking the naphthyl groups provided that (i) the compound is not suramin and (ii) when A is not

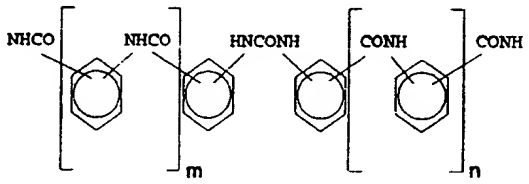


wherein m and n are independently 0, 1 or 2, then at least one of R^1 to R^4 is -OH or an acidic group; or a pharmaceutically acceptable salt, ester, salt of such ester or amide of such compounds, in the manufacture of a medicament for use in treating diseases which benefit from the antagonism of the action of heparin-dependent growth factors.

6. The use of a compound with the structural formula:

(I) 

wherein R^1 to R^4 are each independently one or more of -X, $-N_3$, $-NO_2$, halo, trifluoromethyl, R^5, OR^5, $-CH_2OR^5$, $-OCOR^5$, $-CH_2OCOR^5$, $-NHCOR^5$, $-CH_2NHCOR^5$, $-NR^5R^6$, $-CH_2NR^5R^6$, $-CH_2NO_2$, $CONR^5R^6$, $CH_2CONR^5R^6$, $-COOR^5$, $-CH_2COOR^5$, -CHO and $-CH_2CHO$ and -X is independently $-SO_3R^5$, $-CH_2PO_3R^5R^6$; $-CH_2SO_3R^5$, $-OSO_3R^5$, $-CH_2OSO_3R^5$, $-CH_2OSO_3R^5$, $-NHSO_3R^5$, $-CH_2NHSO_3R^5$, $-OPO_3R^5R^6$, $-CH_2OPO_3R^5R^6$ and $-PO_3R^5R^6$ where R^5 and R^6 are chosen independently from -H and lower alkyl and wherein A is a chemical group comprising at least 5 and no more than 30 bonds directly linking the naphthyl groups provided that (i) the compound is not suramin and (ii) when A is not



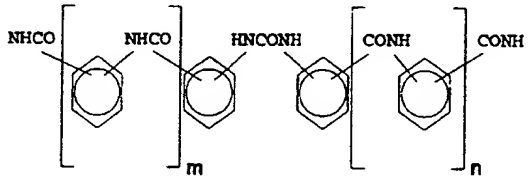
wherein m and n are independently 0, 1 or 2, then at least one of R^1 to R^4 is -OH or an acidic group; or a pharmaceutically acceptable salt, ester, salt of such ester or amide of such compounds, in the manufacture of a medicament for use in treating restenosis.

7. The use of a compound according to any one of the preceding claims wherein the death of cells during treatment of the said disease is *via* apoptosis.

8. The use of a compound according to any one of the preceding claims wherein the linkage of A to the naphthyl ring is *via* an amino group.

9. The use of a compound according to any one of the preceding claims wherein the linkage of A to the naphthyl ring is *via* an amide or sulphonamide group.

10. The use of a compound according to Claim 9 wherein A is:



and m and n are independently 0, 1 or 2.

11. The use of a compound according to any one of Claims 1 to 6 wherein m = n = 0 or 1.

12. The use of a compound according to any one of Claims 1 to 9 wherein A is selected from the group consisting of straight chain or branched alkyl groups, aryl groups, alkylaryl groups, aliphatic dicarboxylic acids, polyenes and derivatives thereof and polyols and derivatives thereof.

13. The use of a compound according to any one of Claims 1 to 9

wherein A is an oligopeptide.

14. The use of a compound according to any one of Claims 1 to 9 wherein A is a carbohydrate.

15. The use of a compound according to any one of Claims 1 to 14 wherein $R^1 = R^4$ and $R^2 = R^3$.

16. The use of a compound according to any one of Claims 1 to 15 wherein R^1 to R^4 are independently selected from the group consisting of -X, -H, lower alkyl, -OH, $-CH_2OH$, $-NHCOCH_3$, $-CH_2NHCOCH_3$, $-CONHCH_3$ and $-CH_2CONHCH_3$ and wherein X is independently selected from the group consisting of $-SO_3H$, $-CH_2SO_3H$, -COOH, $-CH_2COOH$, $-NHSO_3H$, $-CH_2NHSO_3H$, $-OSO_3H$ and $-CH_2OSO_3H$.

17. The use of a compound according to any one of Claims 1 to 16 wherein the naphthyl rings are monohydroxydisulphonate substituted or are dihydroxymonosulphonate substituted.

18. The use of a compound according to any one of the preceding claims wherein there are one, two or three R^1 groups selected independently from -X and -OH and one, two or three R^4 groups selected independently from -X and -OH and R^2 and R^3 are hydrogen or 3-X.

19. The use of a compound according to any one of Claims 1 to 15 wherein one each of the R^1 and R^4 groups is -OH at position 8.

20. The use of a compound according to any one of Claims 1 to 15

wherein one each of the R^2 and R^3 groups is -X at position 3 or 4.

21. The use of a compound according to any one of Claims 1 to 15 wherein one each of the R^1 and R^4 groups are -X at position 6 and one each of the R^2 and R^3 groups are -X at position 3.

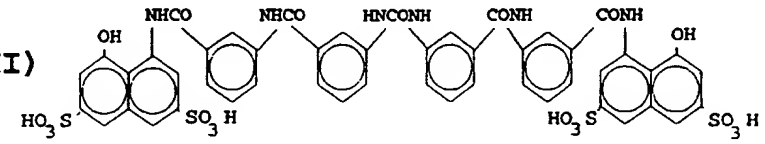
22. The use of a compound according to any one of Claims 1 to 15 wherein two each of the R^1 and R^4 groups are -X at positions 5 and 7.

23. The use of a compound according to Claim 18 and Claims 20 to 22 wherein -X is $-SO_3H$.

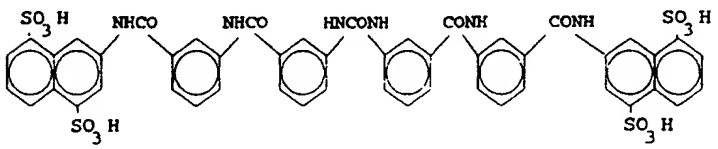
24. The use of a compound according to any one of Claims 1 to 15 wherein R^1 and R^4 are each (8-OH, 6-SO_3H) and R^2 and R^3 are each 3-SO_3H.

25. The use of a compound according to Claim 10 wherein the compound is one of:

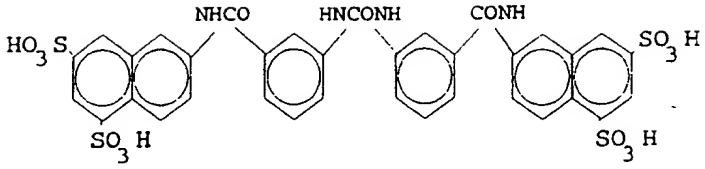
(III)



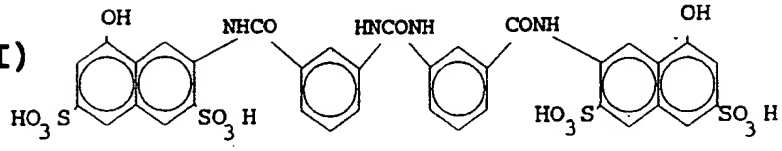
(IV)



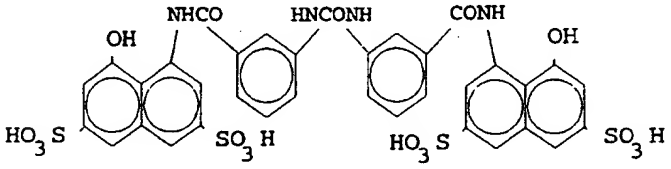
(V)



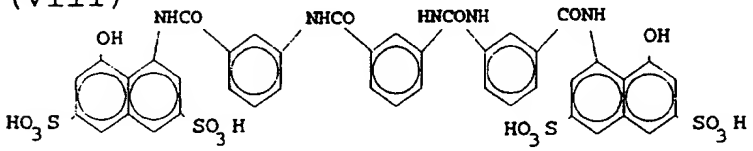
(VI)



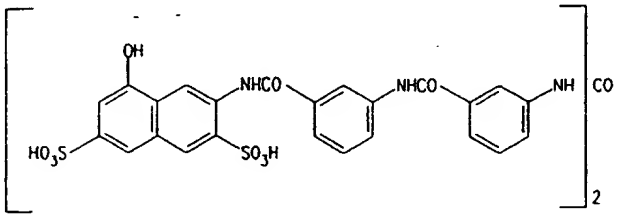
(VII)



(VIII)

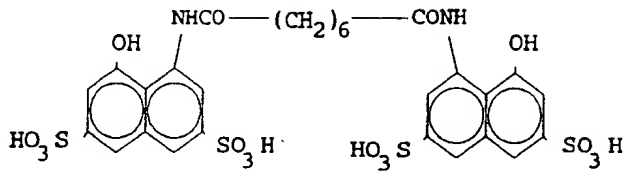


CPD15

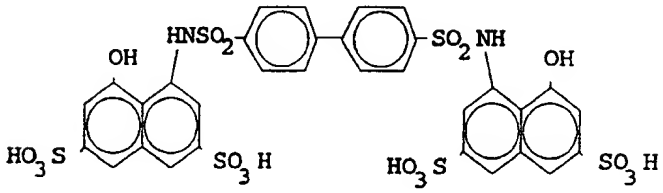


26. The use of a compound according to Claim 12 wherein the compounds is one of:

(IX)



(X)



27. The use of a compound according to Claim 1 wherein the undesired angiogenesis contributes to cancer in humans or other mammals.

28. The use of a compound according to Claim 1 wherein the medicament is for use as a female contraceptive.

29. The use of a compound according to Claim 1 wherein the undesired angiogenesis contributes to diabetic retinopathy.

30. The use of a compound according to Claim 1 wherein the undesired angiogenesis contributes to psoriasis.

31. The use of a compound according to Claim 1 wherein the undesired angiogenesis contributes to rheumatoid arthritis.

32. The use of a compound according to Claim 27 wherein the cancer is hormone-refractory prostate cancer.

33. The use of a compound according to Claim 2 wherein the cancer is hormone-refractory breast cancer.

34. The use of a compound according to Claim 3 wherein the fibrotic disease is selected from the group consisting of pulmonary fibrosis, retroperitoneal fibrosis, scleroderma, cirrhosis of the liver, fibrosing syndromes include mediastinal fibrosis, sclerosing cholangitis, Riedel's thyroiditis, pseudo tumour of the orbit, Peyronie's disease, chronic pancreatitis, Crohn's disease, endocardial fibroelastosis, endomyocardial fibrosis and glomerulonephritis.

35. The use of a compound according to Claim 5 wherein the diseases which benefit from the antagonism are selected from the group consisting of benign prostatic hypertrophy and fibromuscular hyperplasia of large vessels.

36. A method of reducing undesired angiogenesis comprising administering to a mammal having undesired angiogenesis a compound as defined in any one of Claims 1 and 8 to 26.

37. A method according to Claim 36 wherein the undesired angiogenesis contributes to cancer in humans or other mammals.

38. A method of preventing conception comprising administering to a female a compound as defined according to any one of Claims 1 and 8 to 26.

39. A method according to Claim 36 wherein the undesired angiogenesis contributes to retinopathy.

40. A method according to Claim 39 wherein the retinopathy is diabetic retinopathy.

41. A method according to Claim 36 wherein the undesired angiogenesis contributes to psoriasis.

42. A method according to Claim 36 wherein the undesired angiogenesis contributes to a chronic inflammatory disease.

43. A method according to Claim 42 wherein the chronic inflammatory disease is rheumatoid arthritis.

44. A method according to Claim 37 wherein the cancer is selected from the group consisting of prostate cancer, breast cancer and ovarian cancer.

45. A method of treating cancer comprising administering to a mammal having cancer a compound as defined in any one of Claim 1 and 8 to 26.

46. A method according to Claim 45 wherein the cancer is selected from the group consisting of leukaemias, lymphomas and cancers of the uterine cervix, head, neck, brain gliomas, breast, colon, lung, prostate, skin (including Kaposi's sarcoma), mouth, nose, oesophagus, stomach, liver, pancreas and metastatic forms of any of these.

47. A method of treating fibrotic disease comprising administering to a mammal having fibrotic disease a compound as defined according to any one of Claim 1 and 8 to 26.

48. A method according to Claim 47 wherein the fibrotic disease is selected from the group consisting of pulmonary fibrosis, retroperitoneal fibrosis, scleroderma, cirrhosis of the liver, fibrosing syndromes include mediastinal fibrosis, sclerosing cholangitis, Riedel's thyroiditis, pseudo tumour of the orbit, Peyronie's disease, chronic pancreatitis, Crohn's disease, endocardial fibroelastosis, endomyocardial fibrosis and glomerulonephritis.

49. A method of treating diseases which benefit from antagonism of the action of a growth factor comprising administering to a mammal that would benefit from such antagonism a compound as defined in according to any one of Claim 1 and 8 to 26.

50. A method according to Claim 49 wherein the growth factor is a heparin-binding growth factor or a growth factor dependent on a heparin-binding protein.

51. A method according to Claim 50 wherein the diseases which benefit from the antagonism are selected from the group consisting of benign prostatic hypertrophy and fibromuscular hyperplasia of large vessels.

52. A method of treating a patient with a non-malignant hyper-proliferative disease comprising administering to the patient a compound as defined according to any one of Claim 1 and 8 to 26.

53. A method of inhibiting endothelial cell proliferation comprising the step of exposing the cell to a compound as defined in any one of Claim 1 and 8 to 26.

54. A method of inhibiting growth factor-stimulated cell-proliferation comprising the step of exposing the cell to a compound as defined in any one of Claim 1 and 8 to 26.

55. A method according to Claim 54 wherein the growth-factor stimulated cells are stimulated by a growth factor selected from the group consisting of PDGF, TGF-β, IGF-1, FGF, VEGF, HB-GF and pleiotropin.

56. A method of treating a patient with restenosis comprising administering to the patient a compound as defined according to any one of Claim 1 and 8 to 26.

57. A method of inhibiting epithelial cell growth comprising the step of exposing the said cell to a compound as defined in any one of Claim 1 and 8 to 26.

58. A method for screening *in vitro* for a compound which reduces endothelial cell proliferation comprising (1) adding a test compound to endothelial cells, (2) determining the growth rate of the cells and (3) comparing the growth rate with the same cells which have had a compound of the invention added.

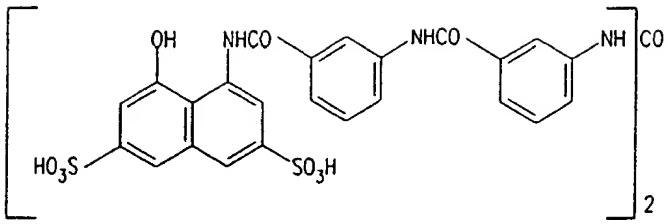
59. A pharmaceutical composition comprising a compound as defined in any one of Claim 1 and Claims 8 to 26 and a female contraceptive agent.

60. A pharmaceutical composition comprising a compound as defined in any one of Claim 1 and Claims 8 to 26 and a cancer therapeutic agent.

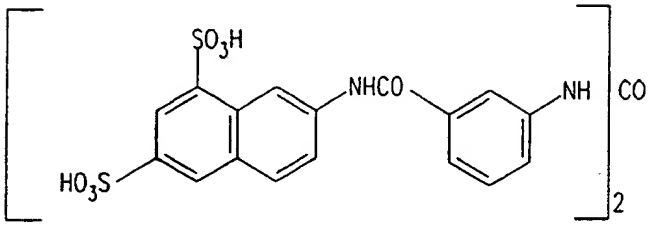
61. A pharmaceutical composition according to Claim 60 wherein the cancer therapeutic agent is selected from the group consisting of nitrogen mustards, ethylenimines, methylmelamines, alkyl sulphonates, triazenes, folic acid analogues, pyrimidine analogues, purine analogues, vinca alkaloids, epipodophyllotoxins, anti-cancer antibiotics, anti-cancer enzymes, interferons, anti-cancer platinum coordination complexes, mitoxantrone, hydroxyurea, N-methyl hydrazine, mitotane, taxol, anthracyclines, aminoglutethimide, and hormone agonists/antagonists such as flutamide or tamoxifen.

FIGURE 1 (page 1 of 5)

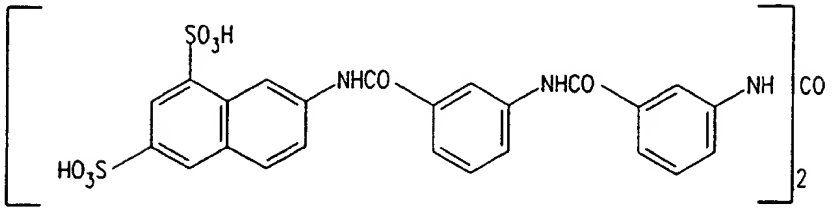
CPD8



CPD9



CPD10



CPD11

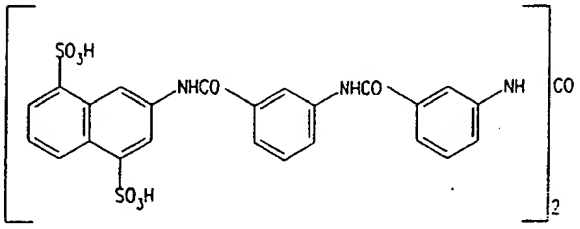
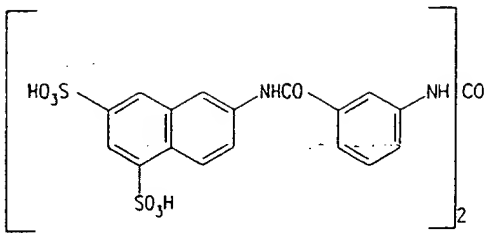
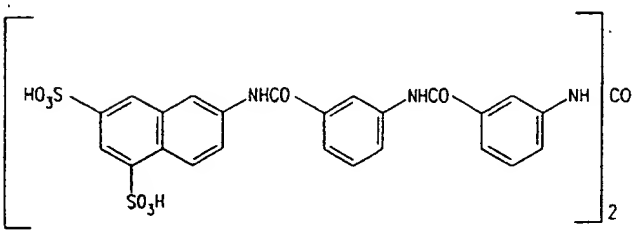


FIGURE 1 (page 2 of 5)

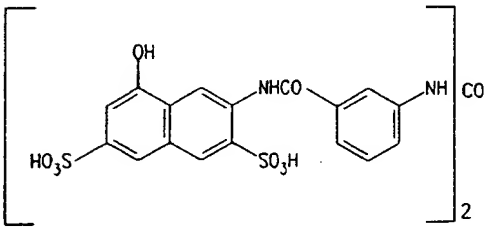
CPD12



CPD13



CPD14



CPD15

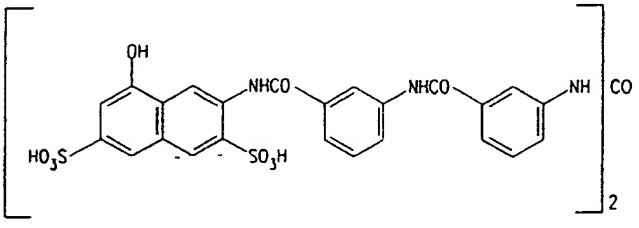
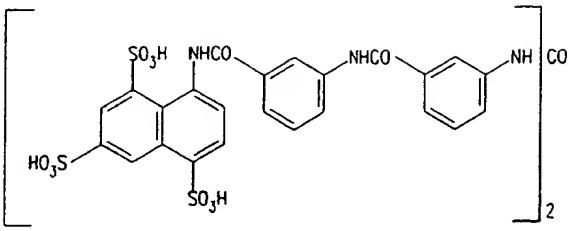
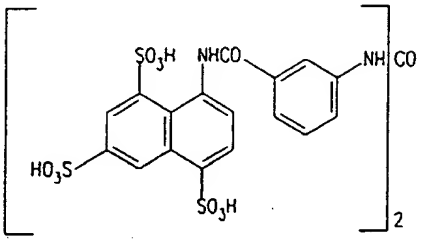


FIGURE 1 (page 3 of 5)

CPD16



N1



N2

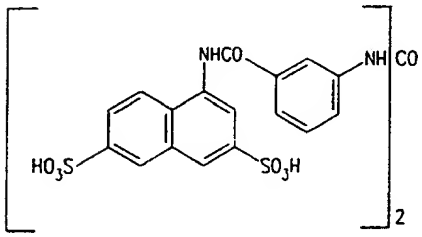
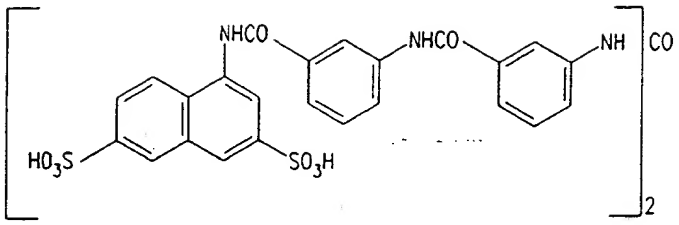
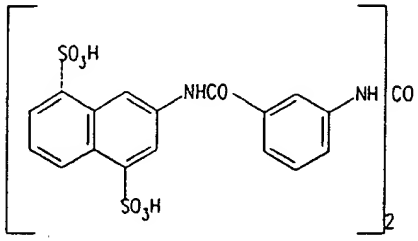


FIGURE 1 (page 4 of 5)

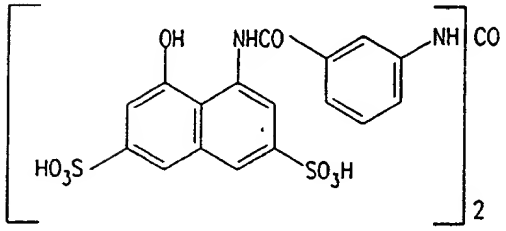
N3



N4



N5



N6

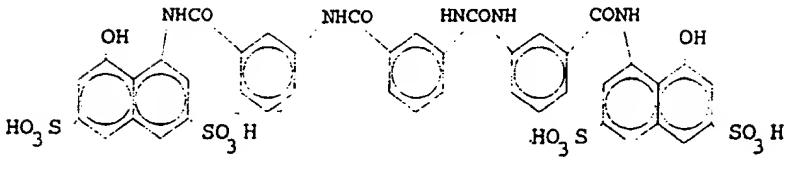
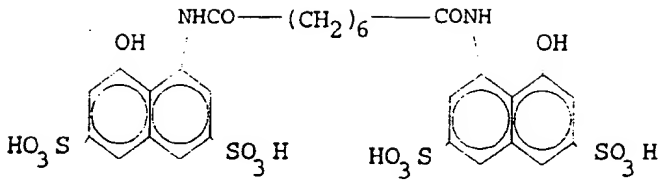


FIGURE 1 (page 5 of 5)

N7



N8

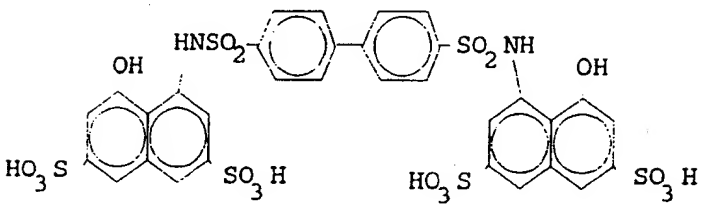
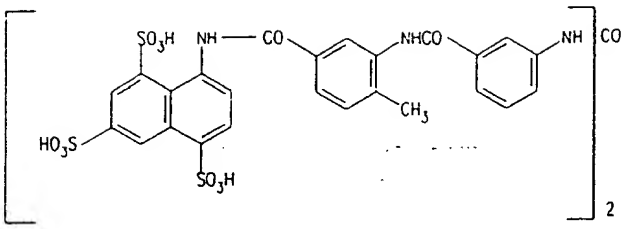
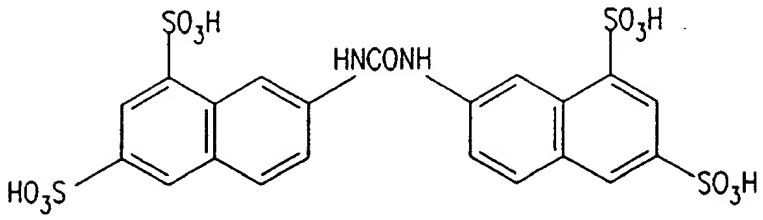


FIGURE 2

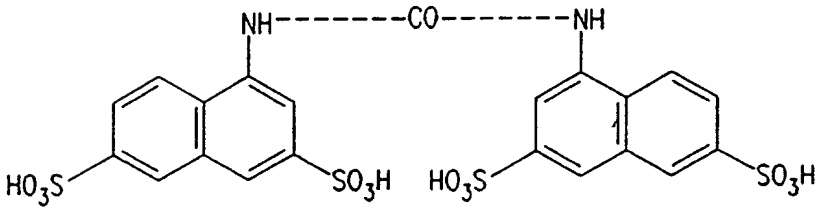
Suramin



CPD1



CDP6



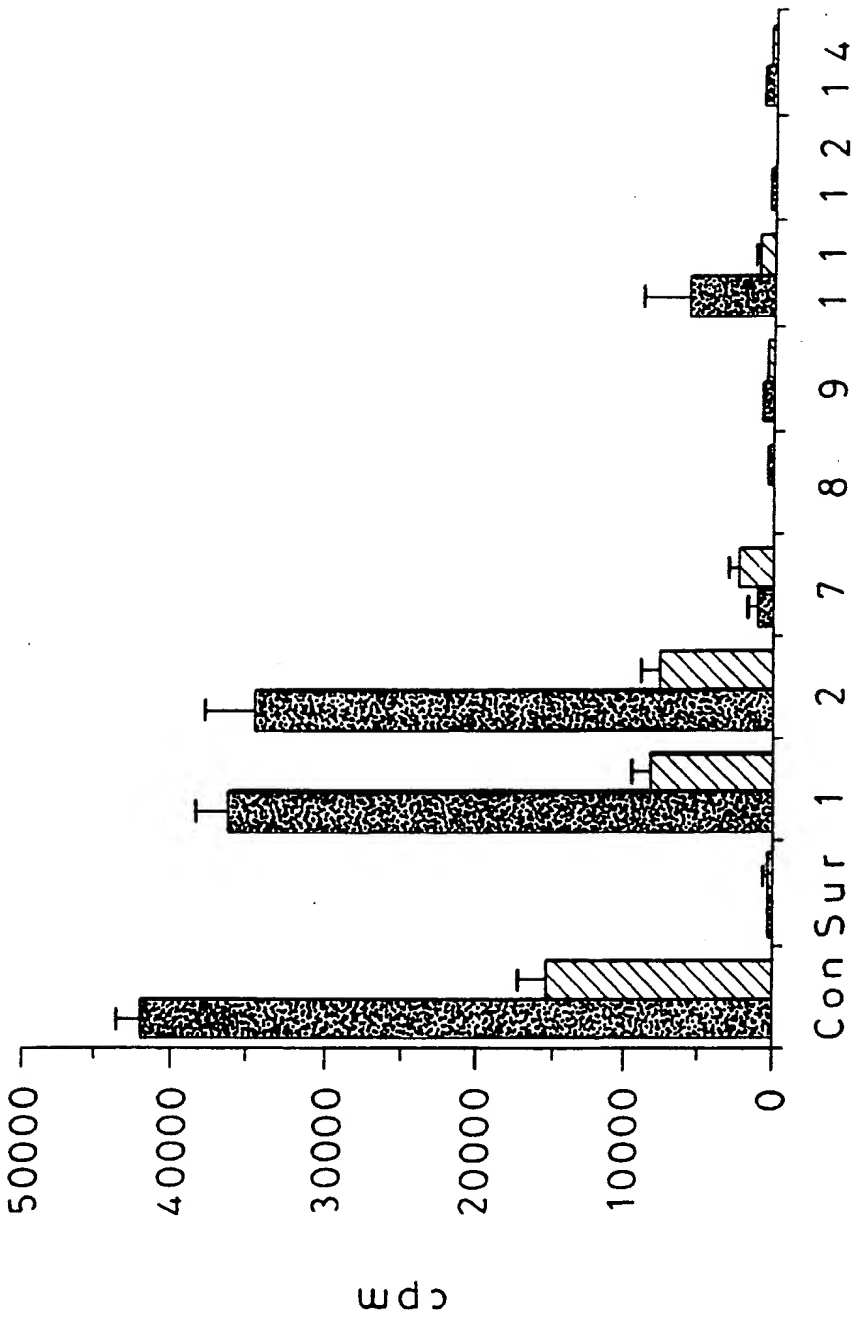
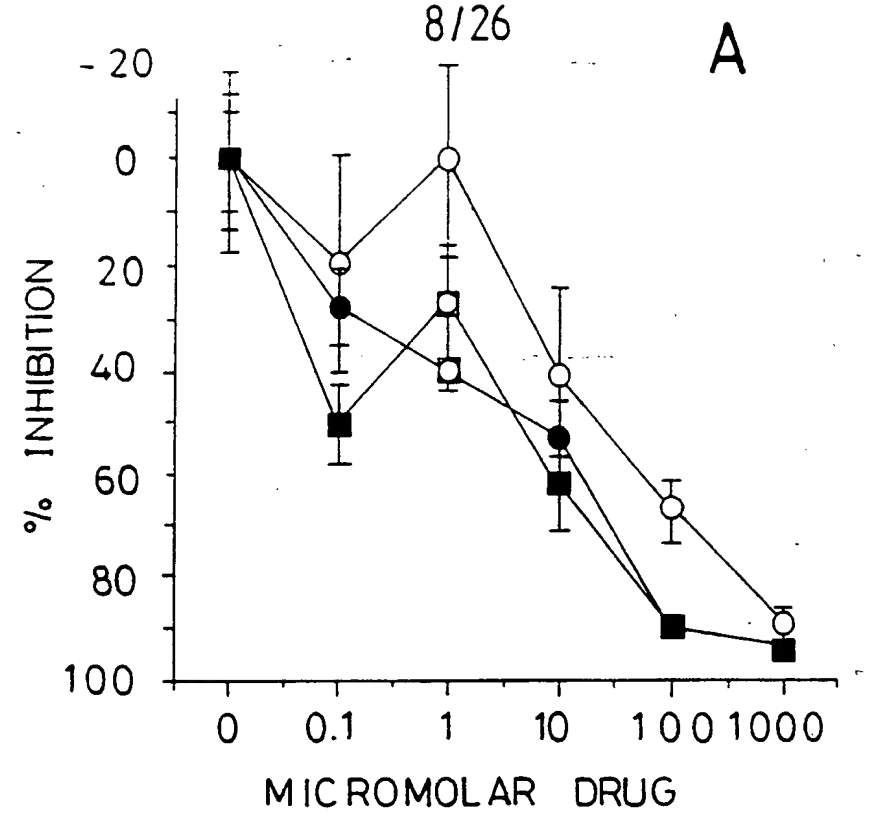


Fig. 3


A
- 20
0
20
40
60
80
100
% INHIBITION
0
0.1
1
10
100
1000
MICROMOLAR DRUG

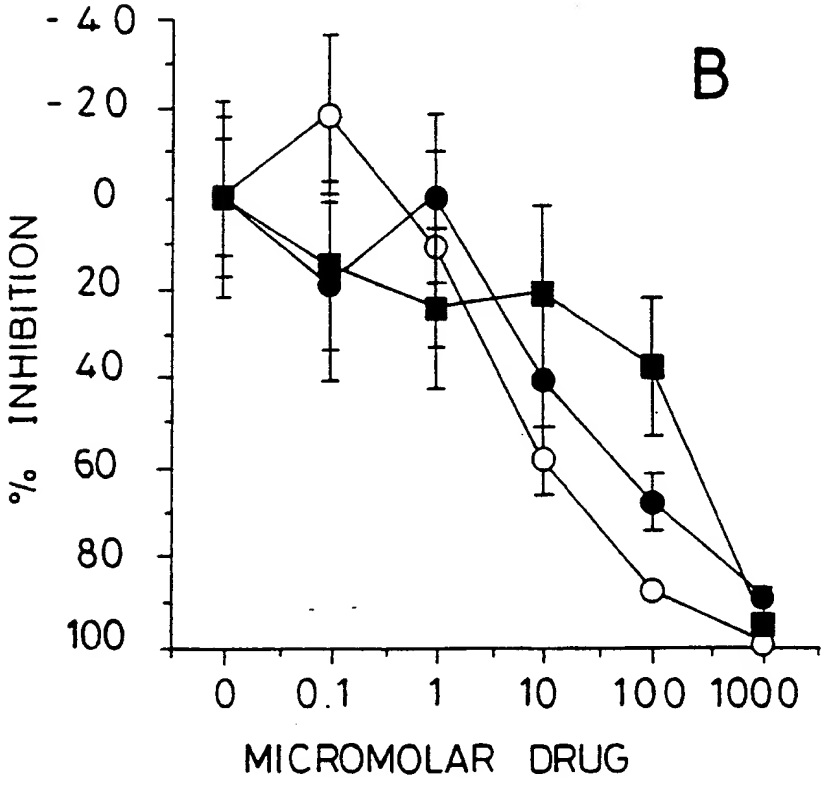

B
- 40
- 20
0
20
40
60
80
100
% INHIBITION
0
0.1
1
10
100
1000
MICROMOLAR DRUG

Fig. 4 (START)

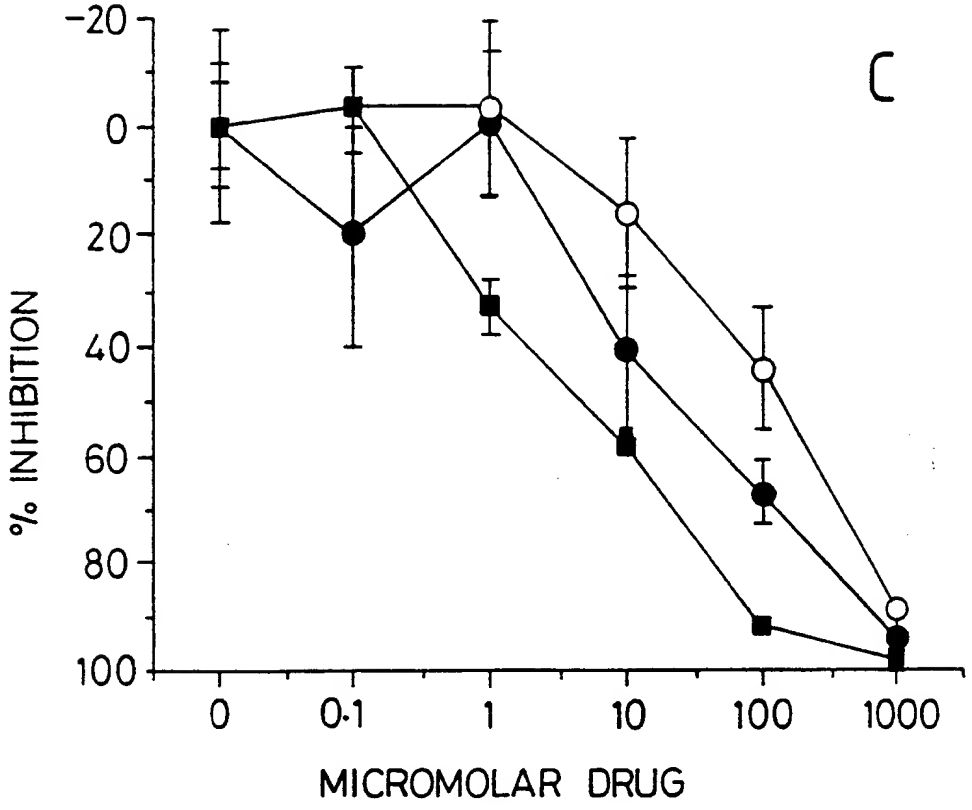

C
-20
0
20
40
60
80
100
% INHIBITION
0
0·1
1
10
100
1000
MICROMOLAR DRUG

Fig. 4 (END)

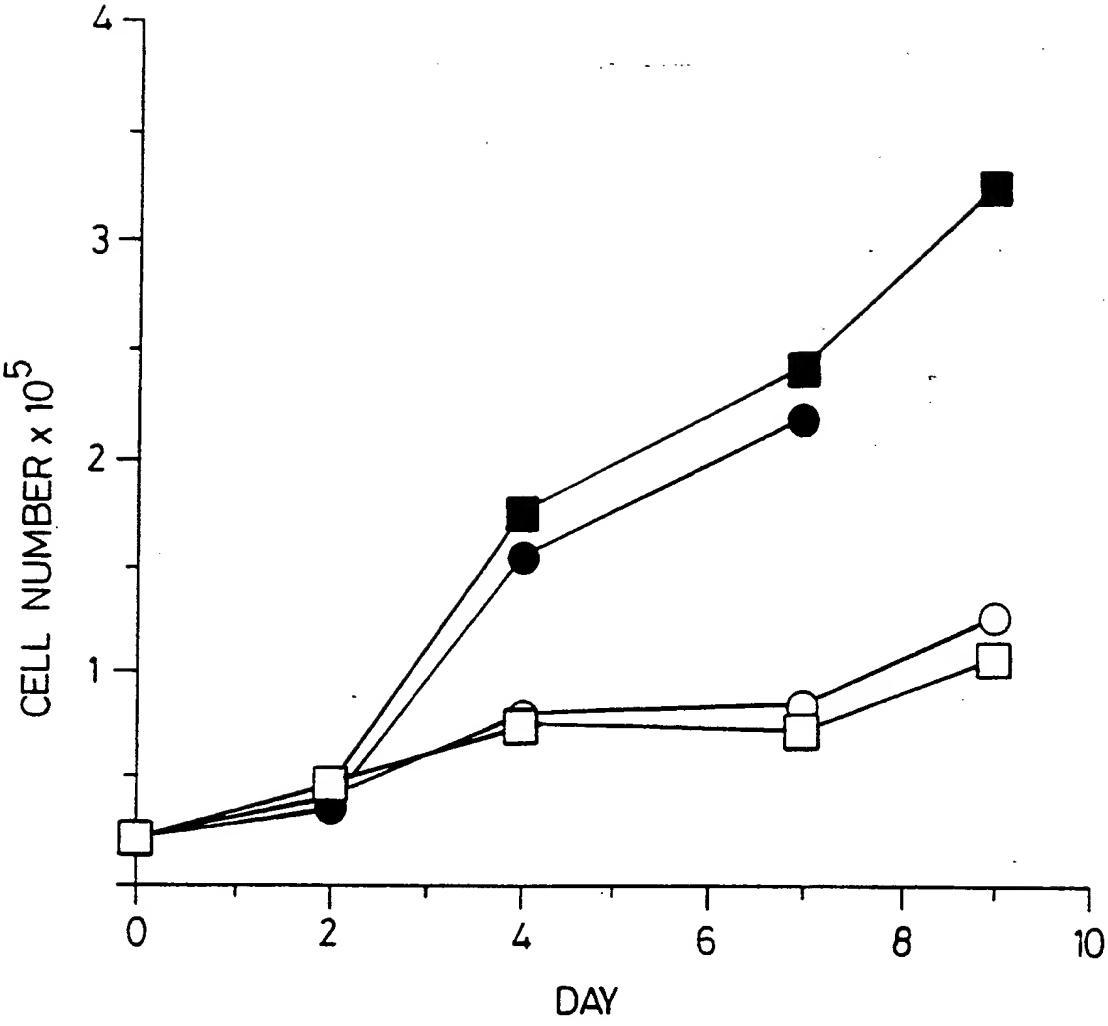


Fig. 5

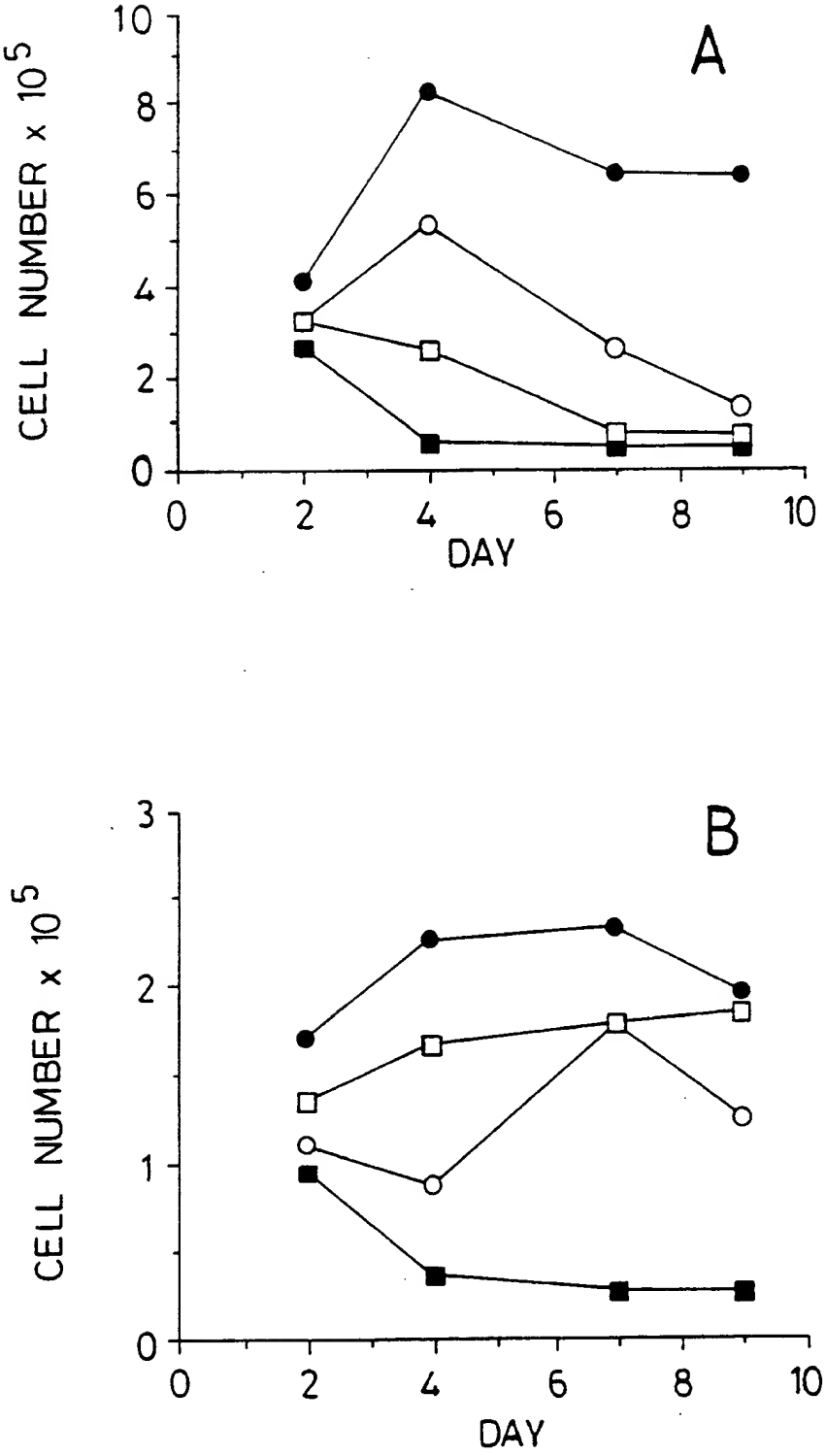
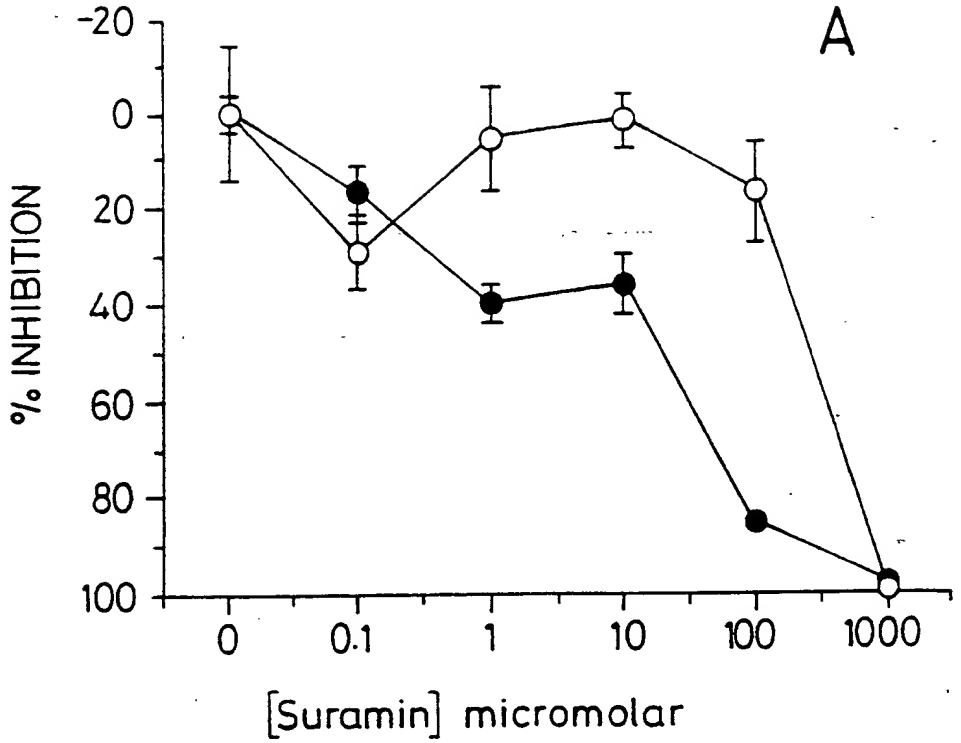

A
CELL NUMBER x 10^5
10
8
6
4
2
0
0
2
4
6
8
10
DAY
B
CELL NUMBER x 10^5
3
2
1
0
0
2
4
6
8
10
DAY

Fig. 6


A
% INHIBITION
-20
0
20
40
60
80
100
0
0.1
1
10
100
1000
[Suramin] micromolar

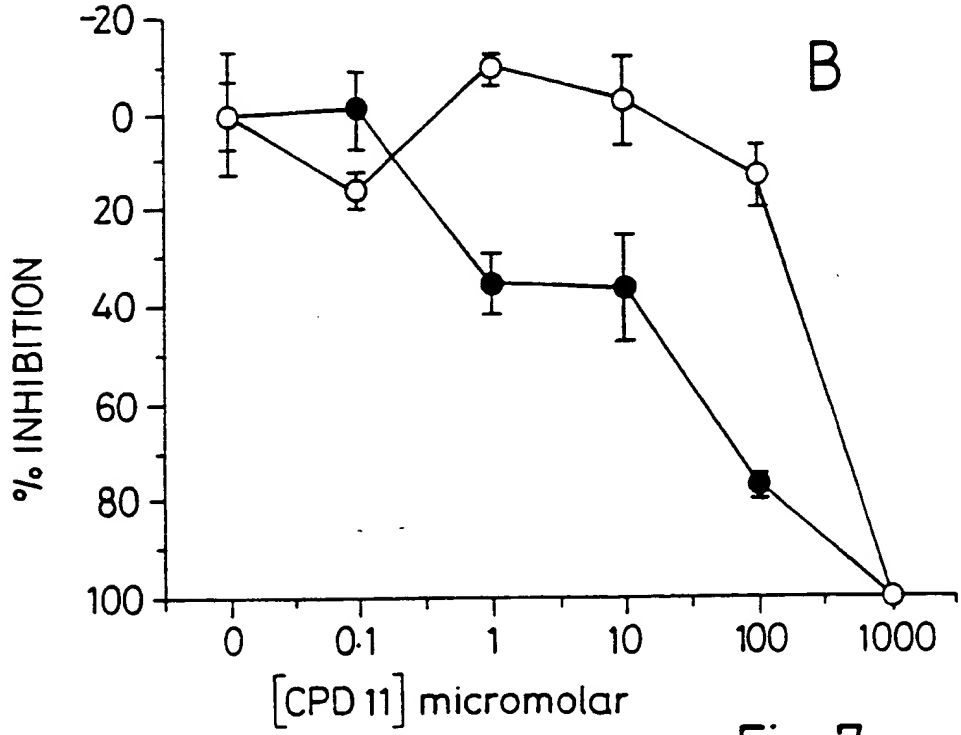

B
% INHIBITION
-20
0
20
40
60
80
100
0
0·1
1
10
100
1000
[CPD 11] micromolar

Fig. 7 (START)

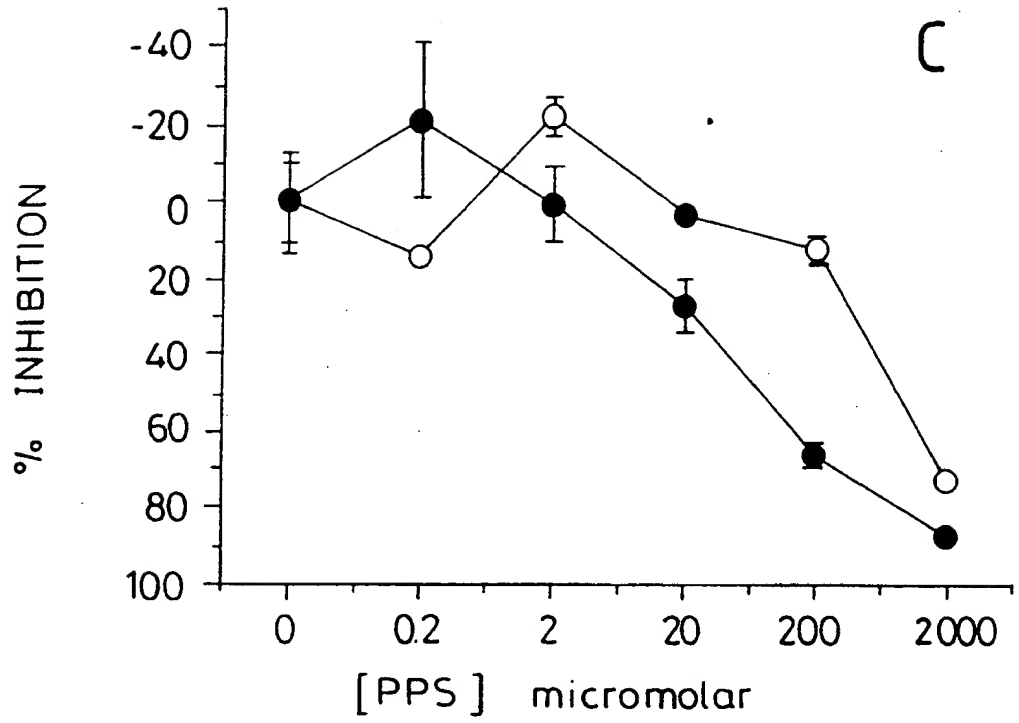

C
-40
-20
0
20
40
60
80
100
% INHIBITION
0
0.2
2
20
200
2000
[PPS] micromolar

Fig. 7 (END)

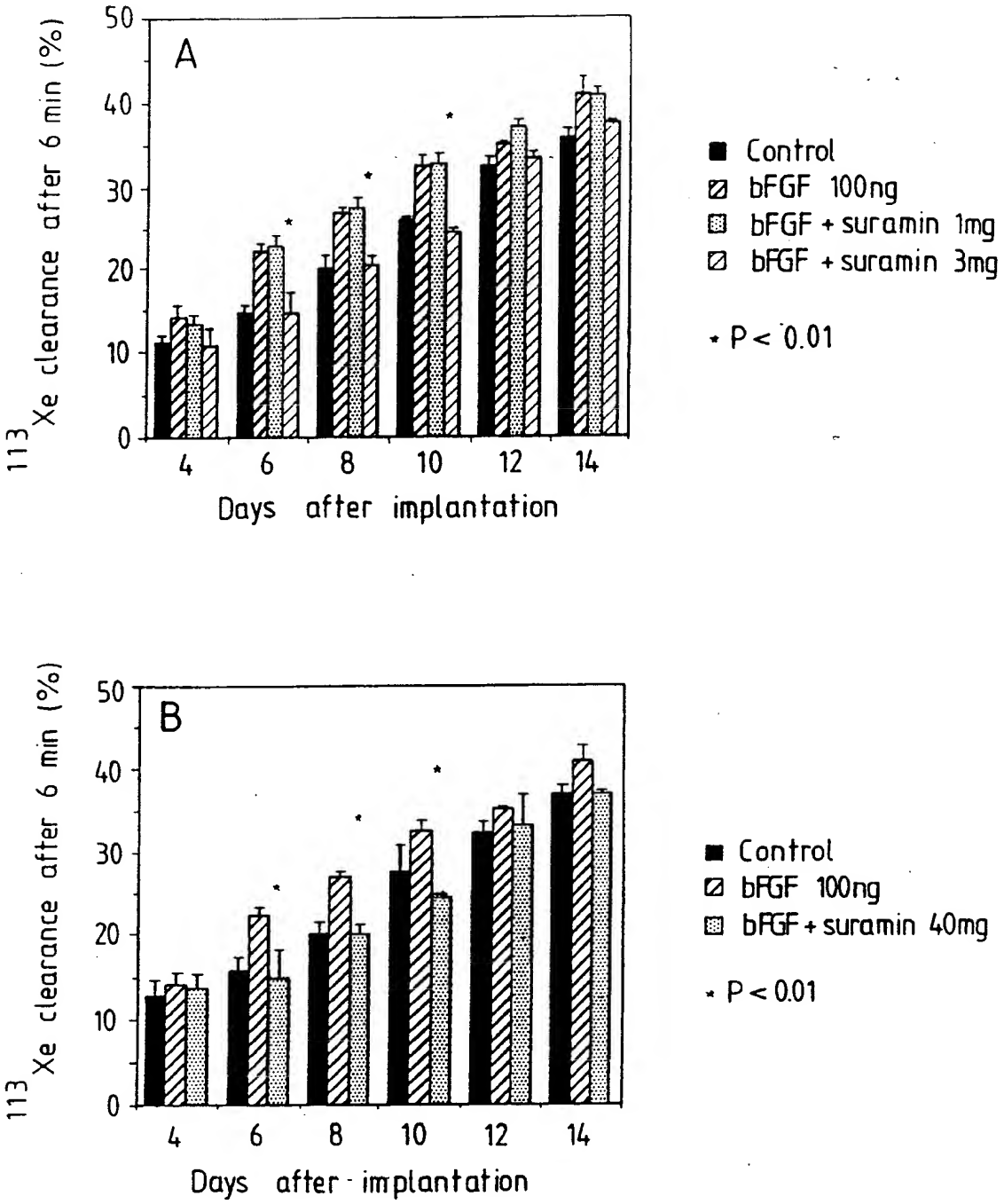

A
B
^{113}Xe clearance after 6 min (%)
0
10
20
30
40
50
4
6
8
10
12
14
Days after implantation
Control
bFGF 100ng
bFGF + suramin 1mg
bFGF + suramin 3mg
bFGF + suramin 40mg
* P < 0.01

Fig. 8(i)

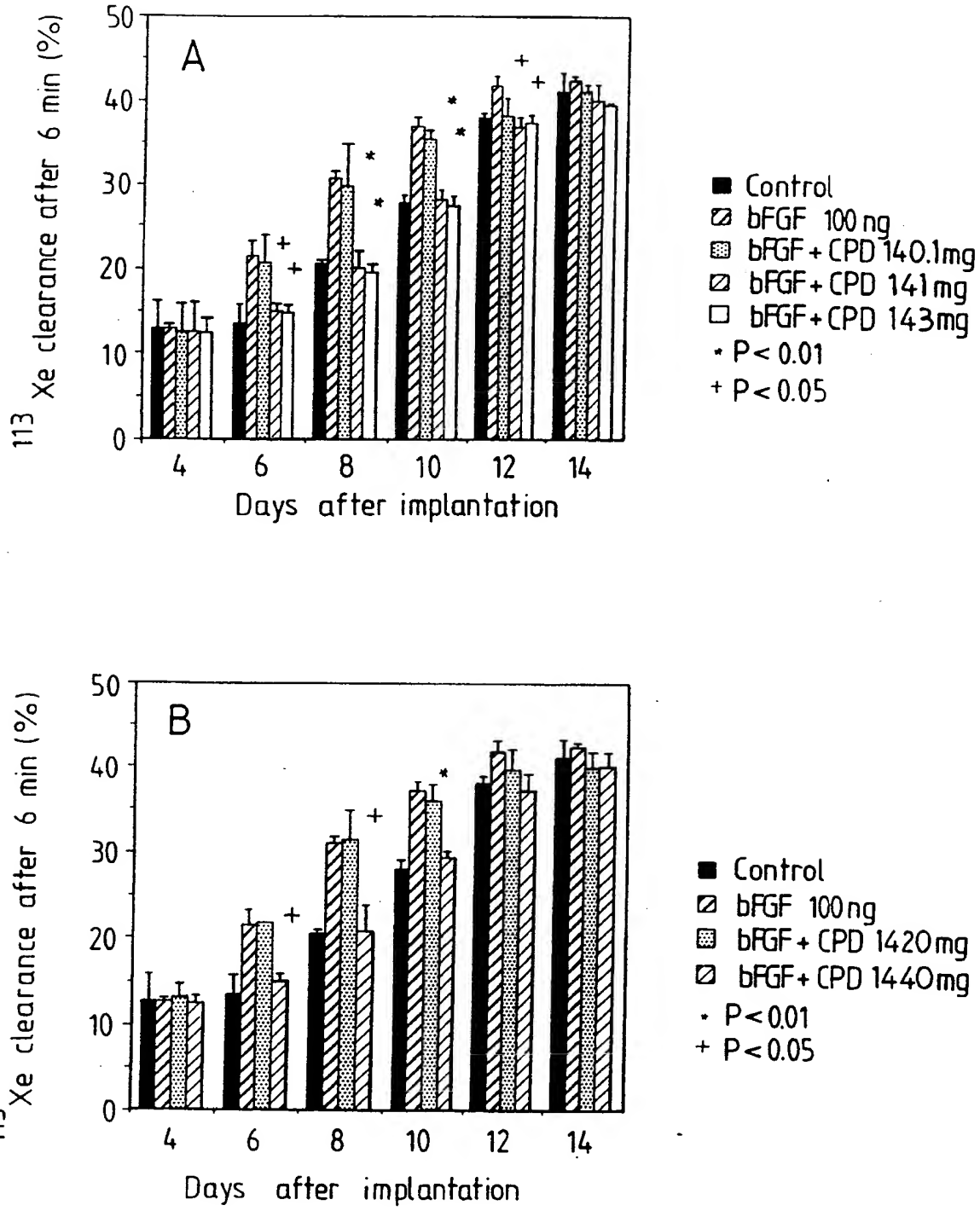

A
B
^{113}Xe clearance after 6 min (%)
Days after implantation
0
10
20
30
40
50
4
6
8
10
12
14
Control
bFGF 100 ng
bFGF + CPD 140.1mg
bFGF + CPD 141 mg
bFGF + CPD 143mg
bFGF + CPD 1420mg
bFGF + CPD 1440mg
* P < 0.01
+ P < 0.05

Fig. 8(ii)

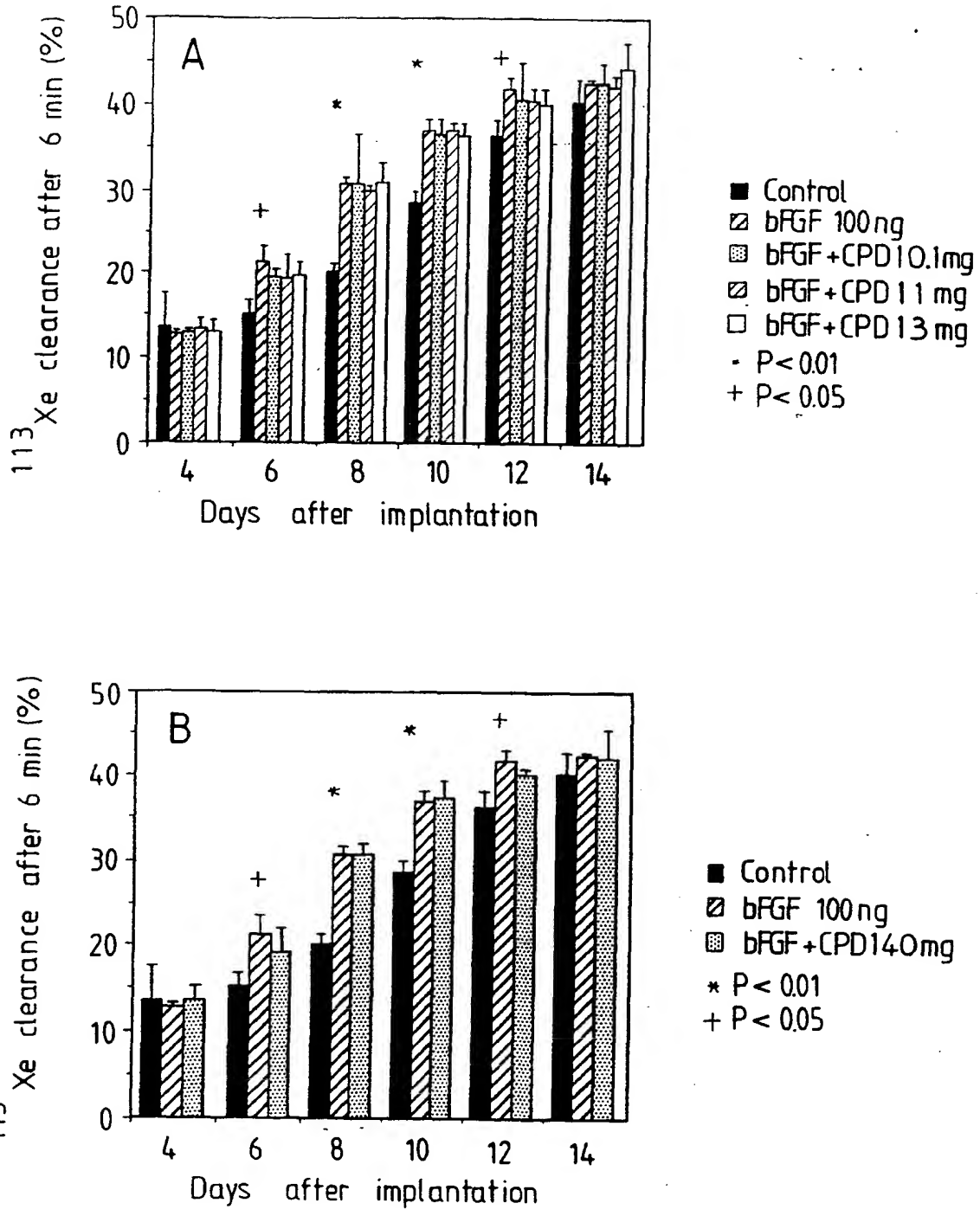
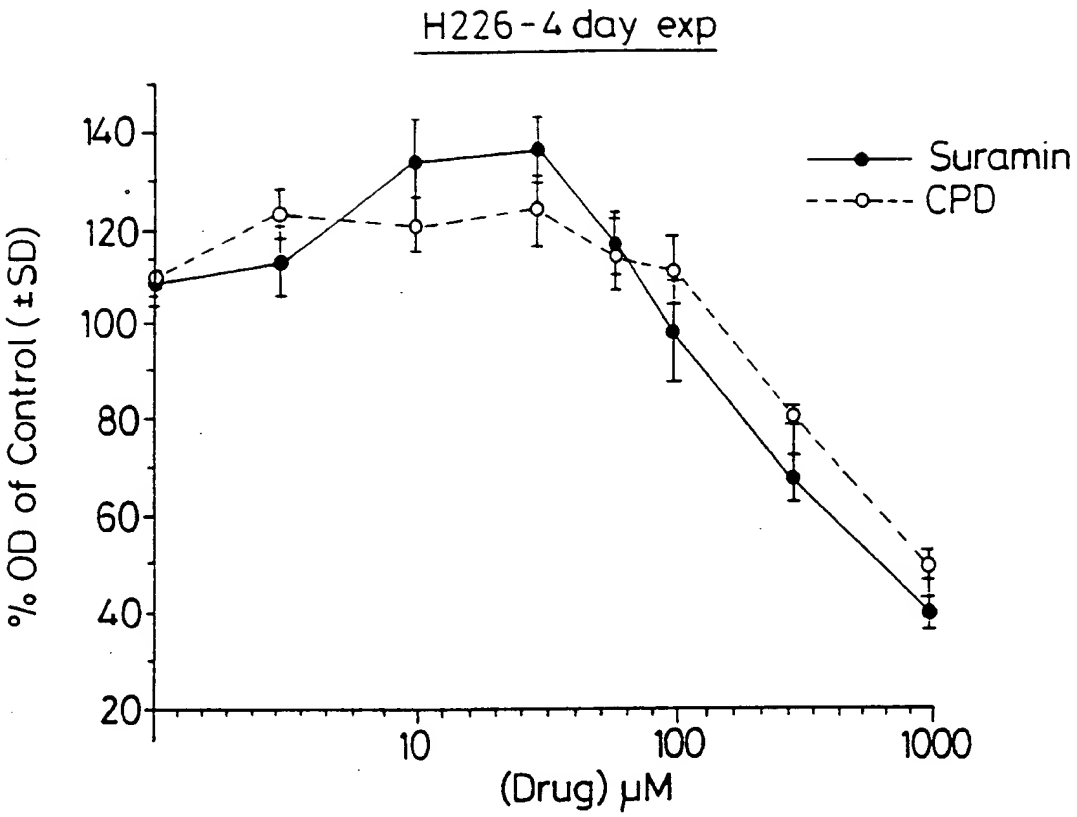

A
B
^{113}Xe clearance after 6 min (%)
Days after implantation
Control
bFGF 100ng
bFGF+CPD10.1mg
bFGF+CPD11mg
bFGF+CPD13mg
bFGF+CPD140mg
- P< 0.01
* P< 0.01
+ P< 0.05

Fig. 8(iii)


H226-4 day exp
140
120
100
80
60
40
20
% OD of Control (±SD)
Suramin
CPD
10
100
1000
(Drug) μM

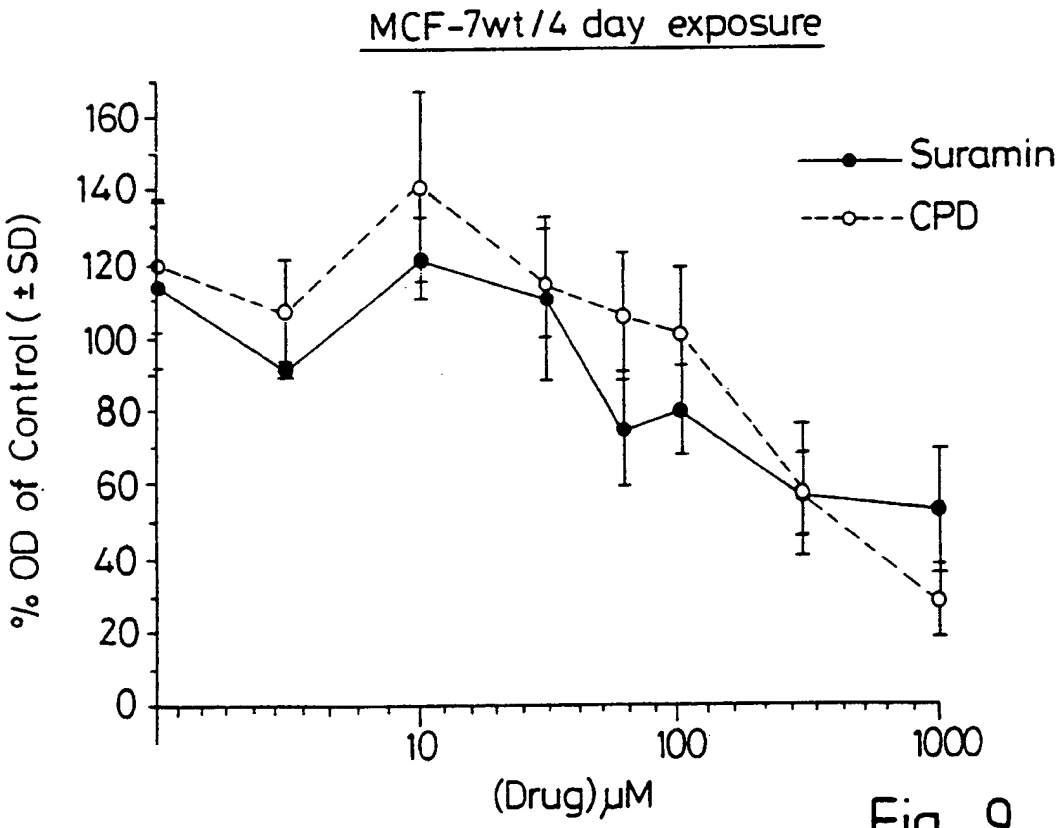

MCF-7wt/4 day exposure
160
140
120
100
80
60
40
20
0
% OD of Control (±SD)
Suramin
CPD
10
100
1000
(Drug) μM

Fig. 9

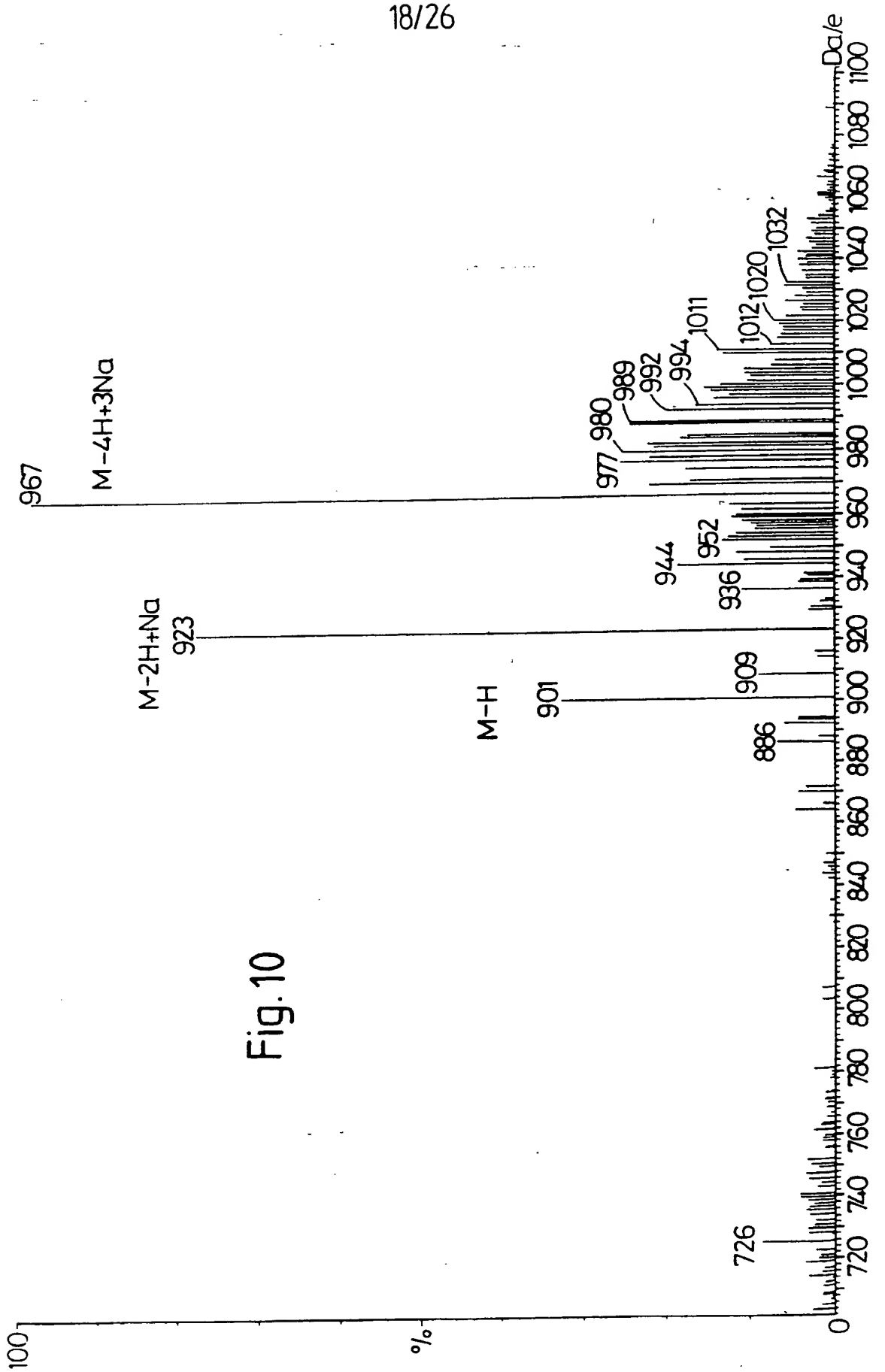

M-4H+3Na
967
977
980
989
992
994
1011
1012
1020
1032
944
952
936
M-2H+Na
923
M-H
901
909
886
726
100
%
0
720
740
760
780
800
820
840
860
880
900
920
940
960
980
1000
1020
1040
1060
1080
1100
Da/e

Fig. 10

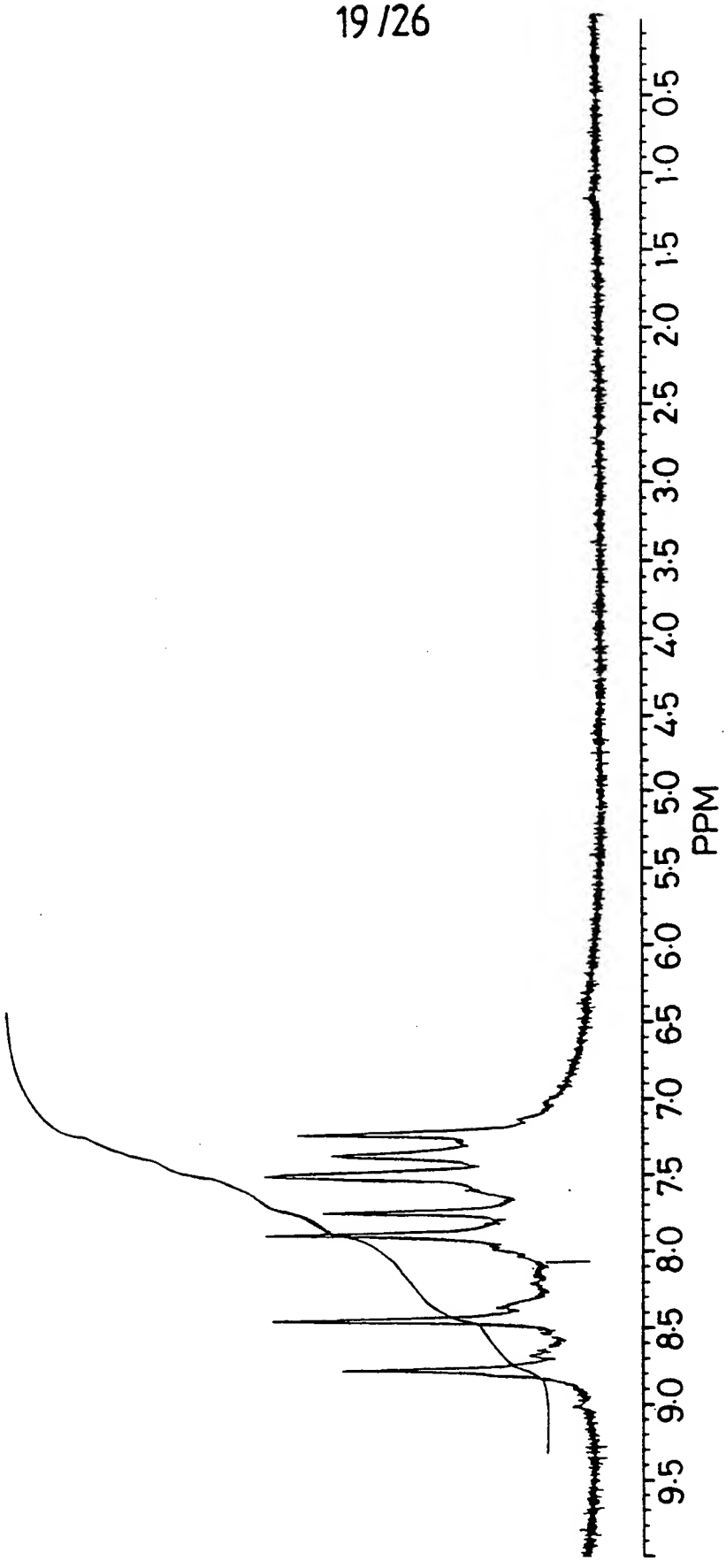


Fig. 11

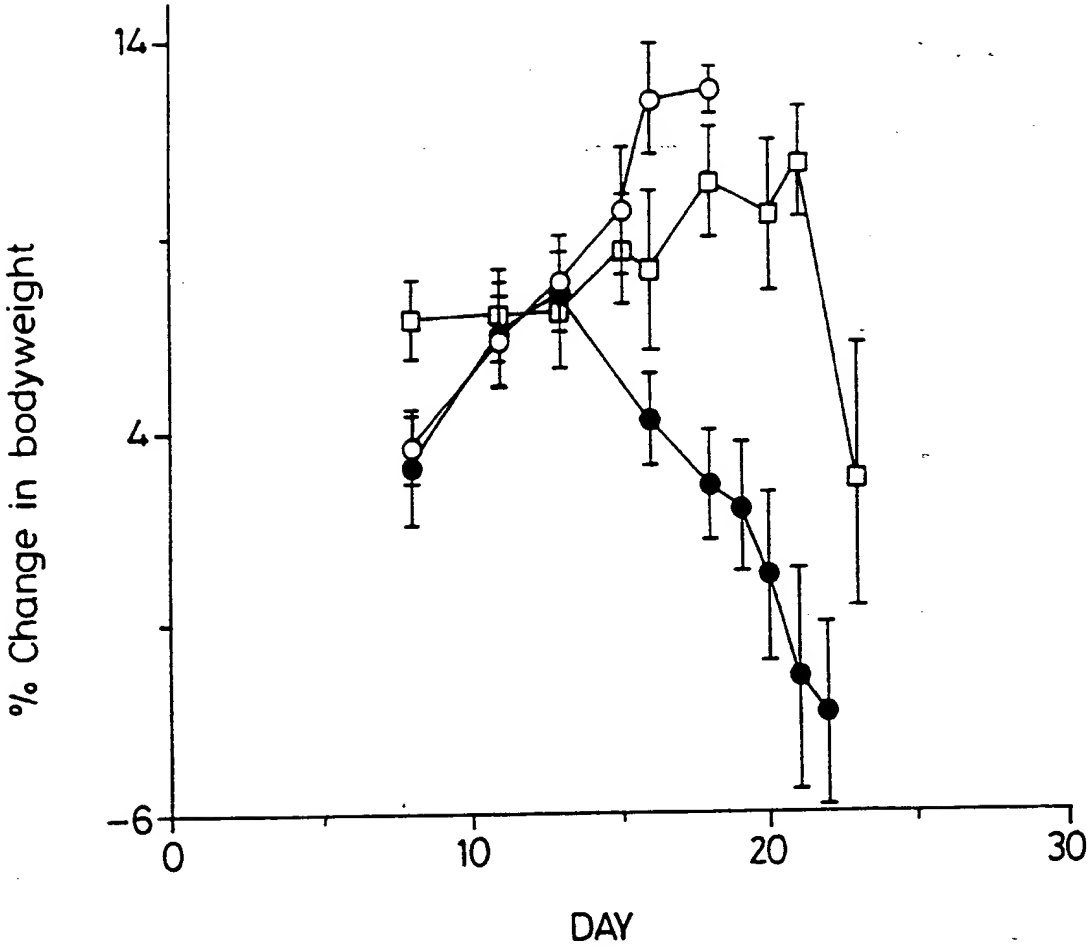


Fig.12

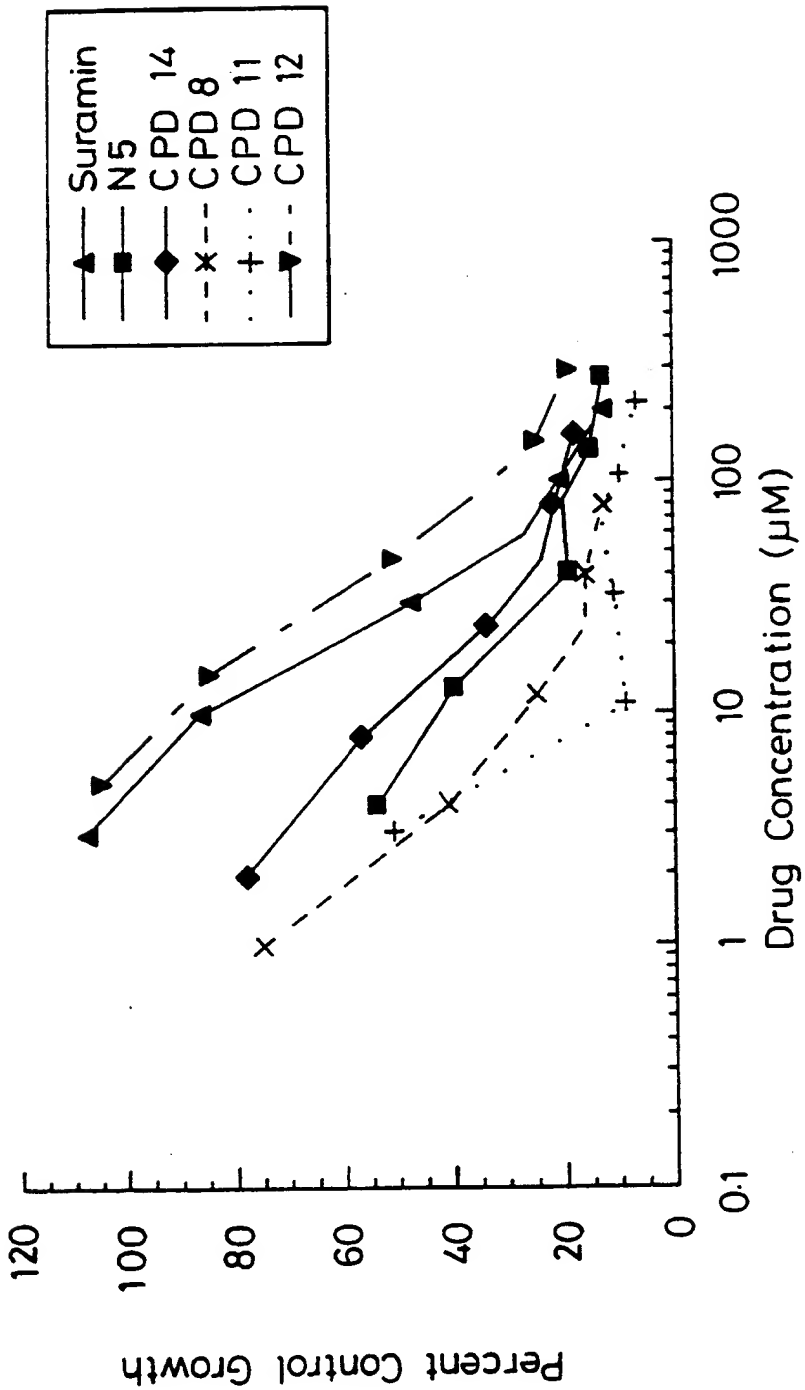

Suramin
N5
CPD 14
CPD 8
CPD 11
CPD 12
Percent Control Growth
120
100
80
60
40
20
0
0.1
1
10
100
1000
Drug Concentration (μM)

Fig.13

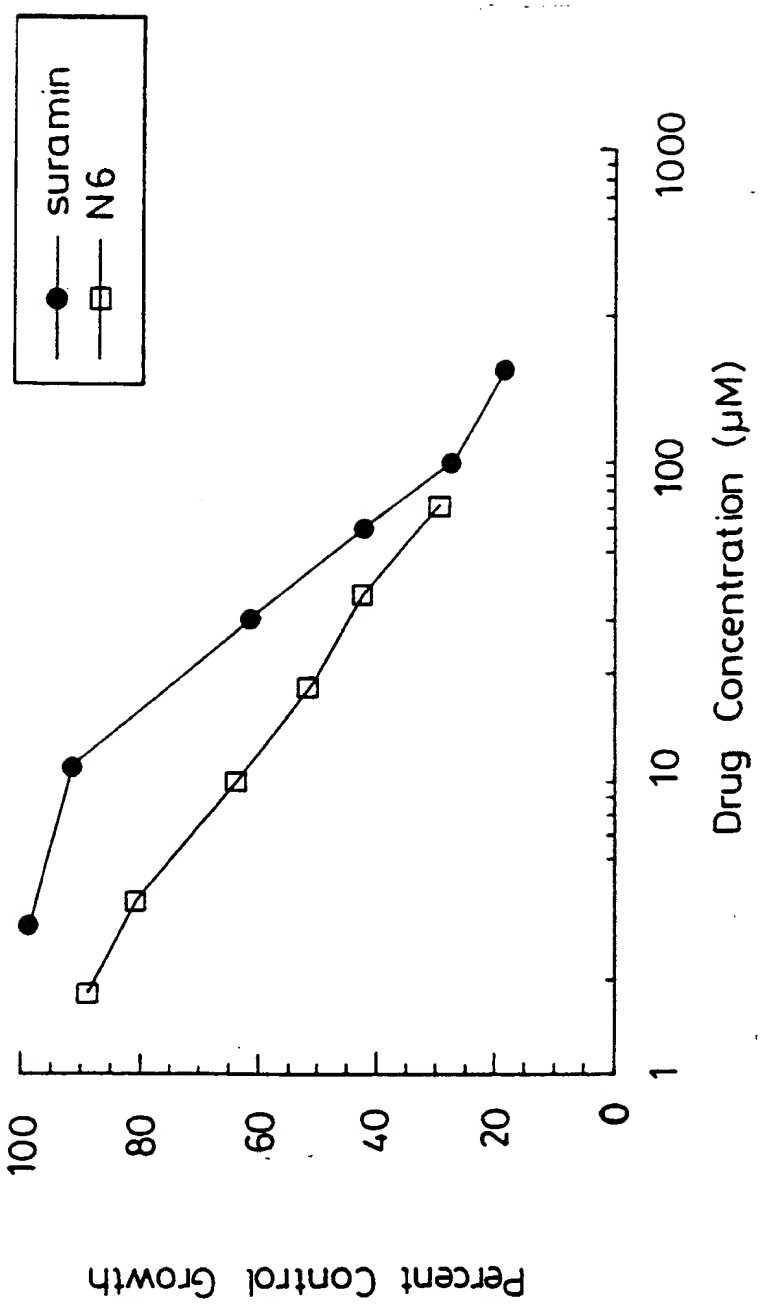

suramin
N6
Percent Control Growth
100
80
60
40
20
0
1
10
100
1000
Drug Concentration (μM)

Fig. 14

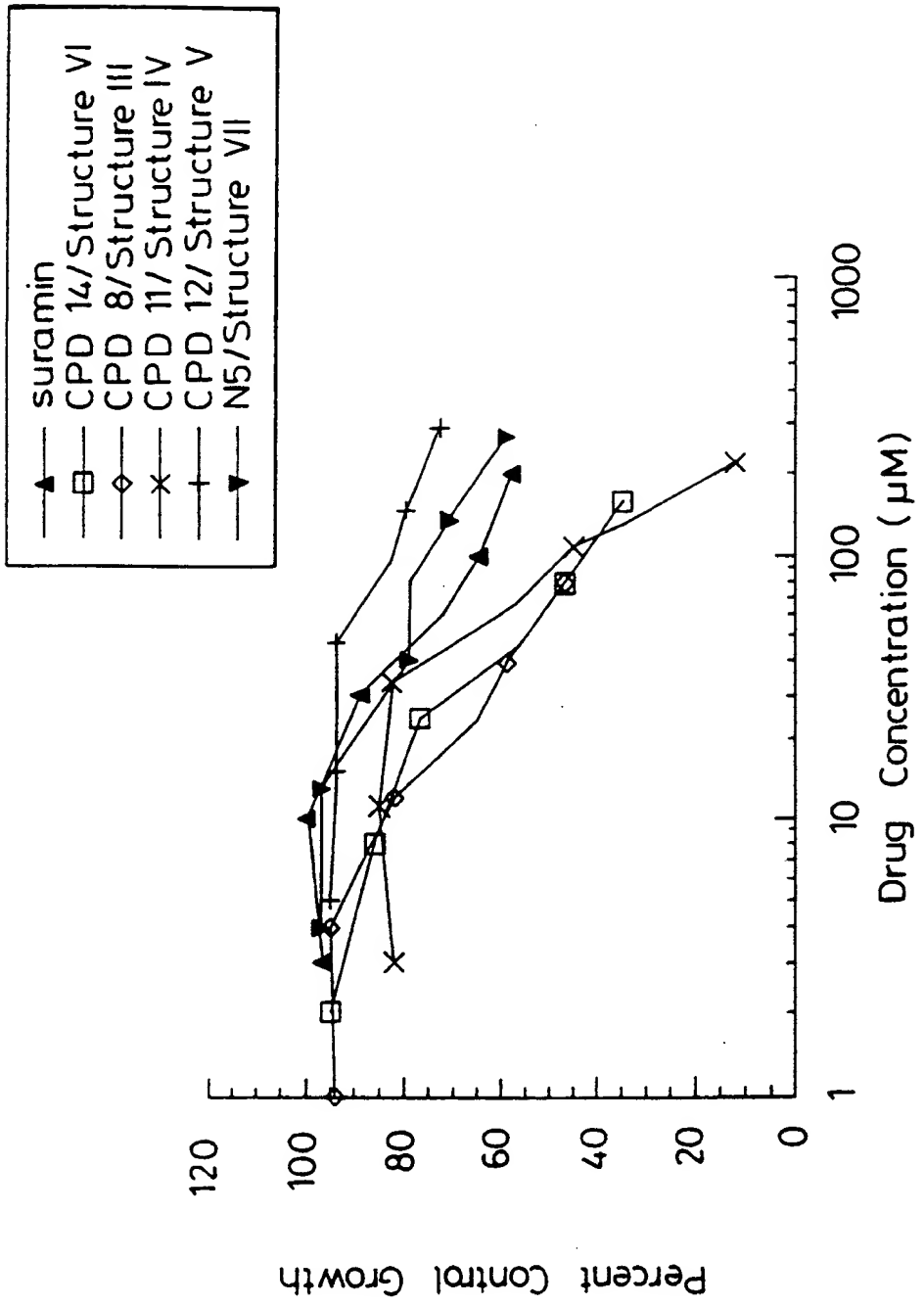

suramin
CPD 14/Structure VI
CPD 8/Structure III
CPD 11/Structure IV
CPD 12/Structure V
N5/Structure VII
Percent Control Growth
120
100
80
60
40
20
0
1
10
100
1000
Drug Concentration (μM)

Fig. 15

Fig. 16

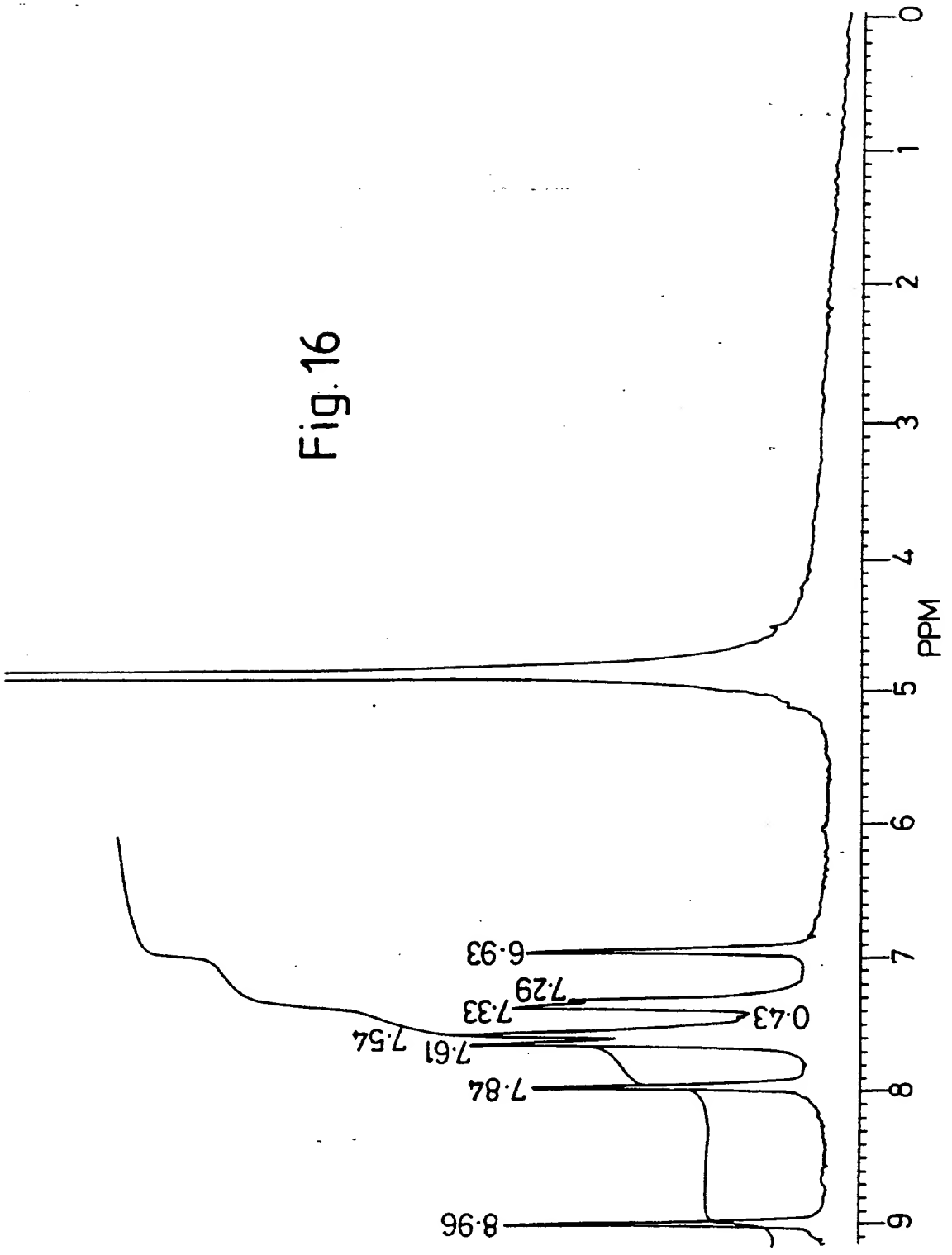
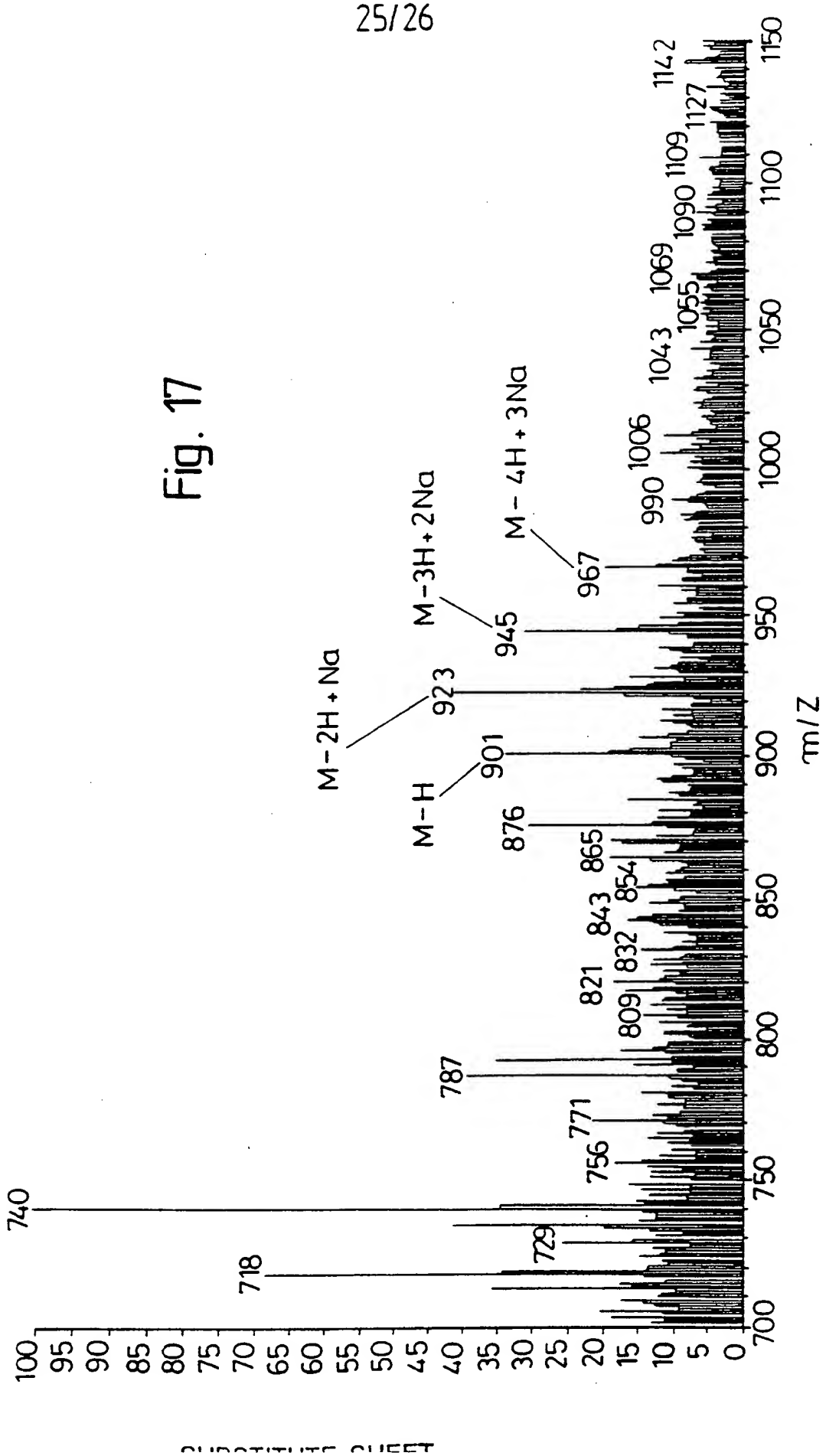


Fig. 17


M-2H+Na
M-3H+2Na
M-4H+3Na
M-H
740
718
729
756
771
787
809
821
832
843
854
865
876
901
923
945
967
990
1006
1043
1055
1069
1090
1109
1127
1142
m/z
100
95
90
85
80
75
70
65
60
55
50
45
40
35
30
25
20
15
10
5
0
700
750
800
850
900
950
1000
1050
1100
1150

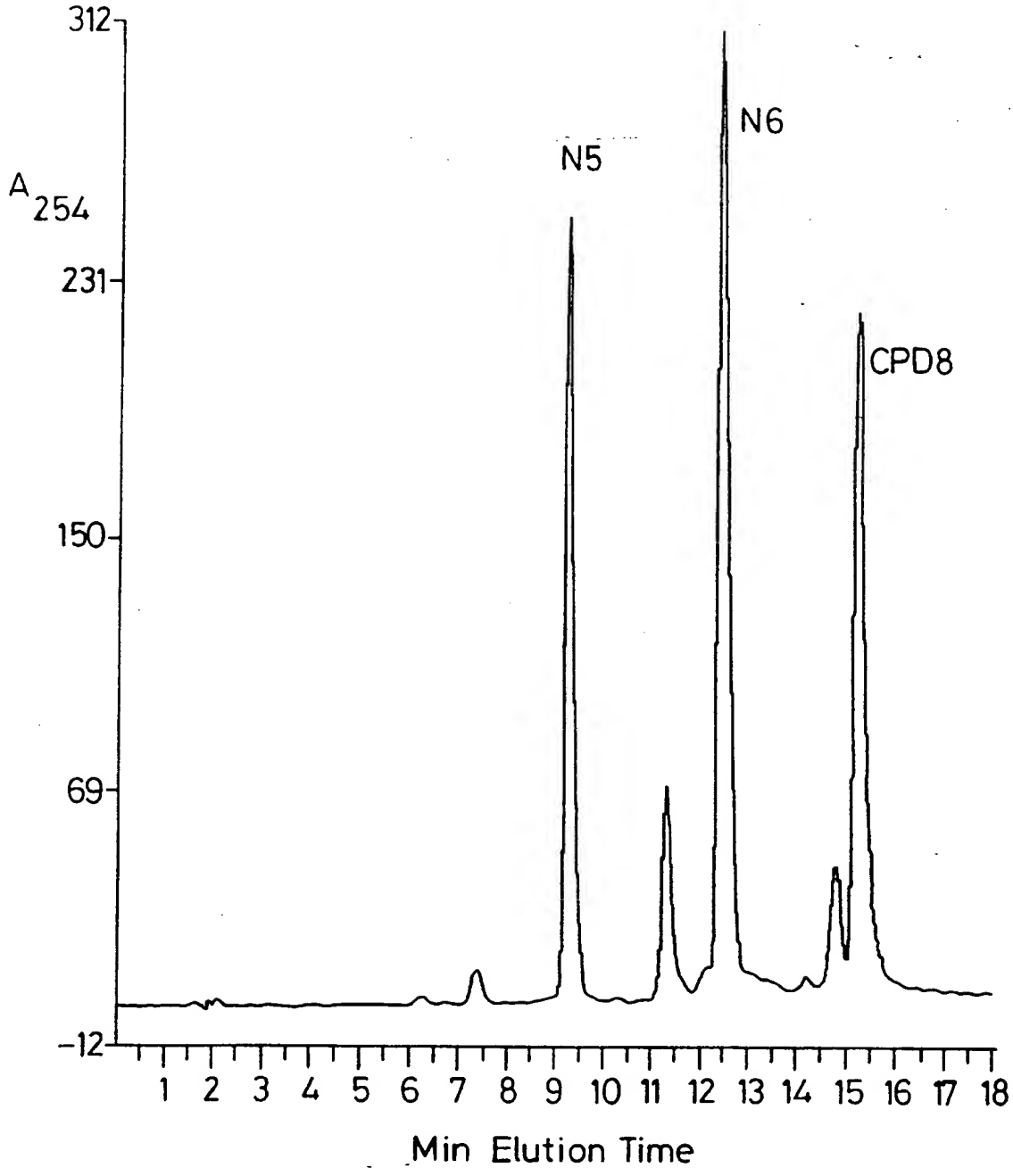

312
231
150
69
-12
A 254
N5
N6
CPD8
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18
Min Elution Time

Fig. 18

PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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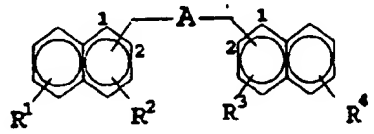
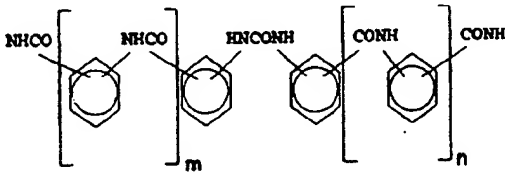
(54) Title: USE OF DINAPHTHALENES COMPOUNDS AS ANTIPROLIFERATIVE AGENTS



(I)



(a)

(57) Abstract

The use of a compound with structural formula (I), wherein R^1 to R^4 are each independently one or more of -X, $-N_3$, $-NO_2$, halo, trifluoromethyl, R^5, OR^5, $-CH_2OR^5$, $-OCOR^5$, $-CH_2OCOR^5$, $-NHCOR^5$, $-CH_2NHCOR^5$, $-NR^5R^6$, $-CH_2NR^5R^6$, $-CH_2NO_2$, $CONR^5R^6$, $CH_2CONR^5R^6$, $-COOR^5$, $-CH_2COOR^5$, -CHO and $-CH_2CHO$ and -X is independently $-SO_3R^5$, $-CH_2PO_3R^5R^6$, $-CH_2SO_3R^5$, $-OSO_3R^5$, $-CH_2OSO_3R^5$, $-CH_2OSO_3R^5$, $-NHSO_3R^5$, $-CH_2NHSO_3R^5$, $-OPO_3R^5R^6$, $-CH_2OPO_3R^5R^6$ and $-PO_3R^5R^6$ where R^5 and R^6 are chosen independently from -H and lower alkyl and wherein A is a chemical group comprising between 5 and 30 bonds directly linking the naphthyl groups provided that (i) the compound is not suramin and (ii) when A is not (a), wherein m and n are independently 0, 1 or 2, then at least one of R^1 to R^4 is -OH or an acidic group or a pharmaceutically acceptable salt, ester, salt of such ester or amide of such compounds, in the manufacture of a medicament for use in reducing undesired angiogenesis, treating cancer, treating fibrotic disease, or treating diseases benefiting from antagonism of the action of fibroblast, vascular endothelial and transforming growth factors. Preferably A is (a) and m and n are independently 0, 1 or 2. Novel pharmaceutical compositions with other medicaments are provided.

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INTERNATIONAL SEARCH REPORT

International Application No
PCT/GB 93/02493

A. CLASSIFICATION OF SUBJECT MATTER
IPC 5 A61K31/165 A61K31/17 A61K31/18 A61K31/00 A61K31/185

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 5 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO,A,91 10649 (FARMITALIA) 25 July 1991 * see claims 1-4,8 and page 11, lines 1-20	1,7-9, 45,60,61
X	WO,A,90 15816 (UPJOHN) 27 December 1990 cited in the application * see page 4 lines 4-27, page 7 lines 27-35, claims 1-3,6,13,14	1,45,60
A	WO,A,92 05153 (AGOURON) 2 April 1992 *see claims 1,42,45 *	1,7-26, 45,46, 60,61
A	EP,A,0 354 818 (HADASSAH) 14 February 1990 * see Claims 1 and 15; Example 4; page 3 line 35 - page 6 line 12 *	1

☐ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

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"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

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Date of the actual completion of the international search
28 March 1994

Date of mailing of the international search report
17. 06. 94

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Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/GB93/02493

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

For further information please see form PCT/ISA/206 dated 21.04.1994.

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searches without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

1,45,46,60,61 and 7-26 partially

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
WO-A-9110649	25-07-91	AU-B-	647446	24-03-94
		AU-A-	7059991	05-08-91
		CN-A-	1053230	24-07-91
		EP-A-	0462258	27-12-91
		JP-T-	4504426	06-08-92
		US-A-	5260329	09-11-93
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		EP-A-	0477195	01-04-92
		JP-T-	4506066	22-10-92
WO-A-9205153	02-04-92	AU-A-	8545091	15-04-92
		CA-A-	2091688	18-03-92
		CN-A-	1062904	22-07-92
		EP-A-	0550566	14-07-93
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